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Third Edition

Concise PATHOLOGY

For Exam Preparation

Geetika Khanna Bhattacharya

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FOR EXAM PREPARATION

THIRD EDITION

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Preface to the Third Edition

Evaluation, a critical and human-error prone component of the medical curriculum, continues to evoke dread in students due to its sometimes "unpredictable" outcome. This book was originally conceived for the students of Pathology, who are naturally apprehensive about this "unpredictability". The enthusiastic response to the previous editions, suggestions received from the student community and the newer learning points now incorporated in standard textbooks, have all necessitated this Edition.

Special effort has been made to include all the latest concepts and changes in the subject including clinical information, wherever feasible, in the chapters to accentuate the relevance of each section to actual patient situations. The text has been presented in a tabulated format, wherever required, to enable easy learning and recall. Definitions and classifications have been incorporated as per World Health Organization and other standard currently accepted guidelines. Many new illustrations and flowcharts have been added to make the book more student-friendly, particularly to the "visual learner". Microphotographs and gross pictures have been introduced in all the chapters to enhance comprehension of the subject. At the same time, every effort has been made to restrict the increase in page extent so that students are not overloaded with information.

In addition, complimentary access to online assessment questions and images along with complete e-book is also provided.

The main objectives of this book remain the same:

- To allow a quick self-assessment and revision before the examination.
- To highlight the "must know" areas (the areas covered in this book are the ones that are frequently referred to/stressed upon by examiners, and are also relevant to the student from knowledge point of view. Proficiency in these topics will aid students' in understanding their clinical subjects in the coming years).
- To enable students to learn and present their knowledge in a format that is easy to appreciate and assess during an examination.

I am certain that this edition is more useful and convenient compared to earlier ones and will find greater acceptability among the undergraduate pathology students.

I would be grateful for any criticism or suggestions which would make this book better in any way. Suggestions and comments from teachers and students can be e-mailed at gtka0612@gmail.com or indiacontact@elsevier.com.

Geetika Khanna Bhattacharya

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Preface to the First Edition

As an examiner and teacher, one often encounters students who have read extensively and have grasped the basic concepts of pathology from their textbooks, but are unable to organize or prioritize this succinctly into the type of answers the examiners expect and appreciate, thus, preventing them from achieving good results/scores in their assessments. The need for a revision or reference manual, which enables students to understand the nuances of key principles of pathology and express them efficiently in the limited time that is usually available to them, thus became obvious.
This book has been written with the following objectives:

- To allow a quick self-assessment and revision before the examination.
- To highlight the areas that need focus (must know areas) and are relevant to the student from both knowledge and examination point of view. (The areas covered in this book are the ones that are frequently referred to/stressed upon by examiners. Also, proficiency in these topics will aid the students in understanding their clinical subjects in the coming years.)
- To enable students to learn and present their knowledge in a format that is easy to appreciate and assess, in the limited time available, during an examination. This book has been primarily written for the discerning undergraduate (MBBS, BDS, and Nursing); however, some topics are covered more extensively, and may benefit the postgraduate as well. I welcome any criticism and suggestions that would contribute towards enhancement of this book.

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Acknowledgements

I am both delighted and humbled by the most enthusiastic reception of the first and second editions of this book and am immensely grateful to my students for all their valuable insights and suggestions, which I have tried to incorporate in this edition. Their overwhelming response keeps me going and makes the entire effort worthwhile.

I would not have been able to complete this all encompassing project without the tremendous patience and endurance of my daughter, the valued support and guidance of my husband, the blessings of my parents, and the constant encouragement and motivation of my brother.

I would like to offer my sincere appreciation to my friends, particularly Dr Sonal Sharma, who has always very generously helped me whenever required. I am also grateful to my departmental staff for their unquestioning support and cooperation.

I am extremely thankful to my publisher, RELX India Pvt. Ltd., particularly Shabina Nasim, Sr Manager–Education Solutions; and Goldy Bhatnagar, Sr Content Development Specialist, for overseeing this project with great commitment and efficiency while conceding to my requests for changes and amendments till the last minute. It has been an absolute pleasure working with them.

Last but not the least I continue to be indebted to the authors of the various publications and reference books that I have consulted during the compilation of this book and regret being unable to acknowledge them all, individually.

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Online University and PGMEE Patterned MCQs MCQ **and SAQs** SAQ

Chapterwise Important Images

SECTION I

General Pathology

1

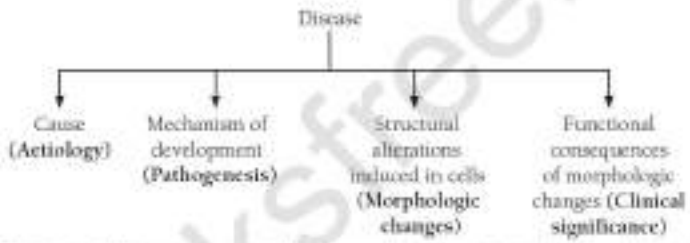
Cell Injury and Cell Death

Q. Define pathology.

Ans. Pathology is the branch of medical science that deals with the study of morphologic (structural) changes caused by disease in cells, tissues, organs, body fluids or whole body (autopsy pathology). It is derived from the words logos (study) and pathos (suffering).

Q. What are the four aspects of a disease that form the core of pathology?

Ans. Disease process is studied under different headings as shown in Flowchart 1.1.



FLOWCHART 1.1. Different aspects that form the core of pathologic basis of disease.

Q. What are the two main branches of pathology?

Ans. The two main branches of pathology are

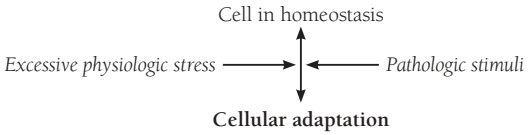
1. **General pathology**: Study of basic, common reactions of cells and tissues to abnormal stimuli that underlie all diseases, eg, response to acute inflammation which is similar irrespective of the type of tissue.
2. **Systemic pathology**: Study of specific responses of specialized organs and tissues to abnormal stimuli, eg, response to organ-specific diseases like myocardial infarction.

Q. Define homeostasis.

Ans. When a cell is able to handle the normal physiologic demands, maintaining a steady state, it is said to be in homeostasis.

Q. Define cellular adaptation.

Ans. Excessive physiologic stress or pathologic stimuli bring about reversible functional and morphologic changes, pushing a normal cell into an altered, but steady state called *cellular adaptation* (Flowchart 1.2).



FLOWCHART 1.2. Cellular adaptation.

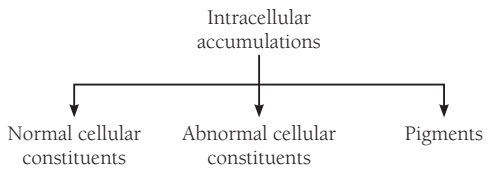
Q. Define cell injury.

Ans. When the cell cannot adapt anymore or when the limits of adaptive response to a stimulus are exceeded, a sequence of events labelled *cell injury* follows.

Q. Enumerate the various cellular responses to injury.

Ans. Cellular responses to injury may manifest as

1. **Cellular adaptations**: Include atrophy, hypertrophy, hyperplasia and metaplasia.
2. **Cell injury**: Sublethal or chronic injurious stimuli can cause (a) 'reversible and irreversible injury' (the latter may lead to cell death by necrosis or apoptosis) and (b) 'subcellular alterations' (residual effects of cell injury).
3. **Intracellular accumulations:** Sublethal or chronic injurious stimuli as well as metabolic derangements can cause intracellular accumulation of normal cellular constituents, abnormal cellular constituents or pigments (Flowchart 1.3).



FLOWCHART 1.3. Intracellular accumulations.

4. **Cell ageing**: Represents progressive accumulation over the years of sublethal injury that manifests with either cell death or inadequate response of the cell to injury. Ageing is influenced by genetic factors, diet and social environment as well as diseases like atherosclerosis, diabetes and osteoarthritis.

Q. What are the different types of cell injuries?

Ans. Types of cell injuries:

1. **Reversible**: If the structural and functional changes, induced by an injurious stimulus, *can* revert to normal on removal of the same, it is called reversible injury (Fig. 1.1).
2. **Irreversible**: If the structural and functional changes, induced by an injurious stimulus, *cannot* be reversed even after removal of the same, it is called irreversible injury (Fig. 1.1).

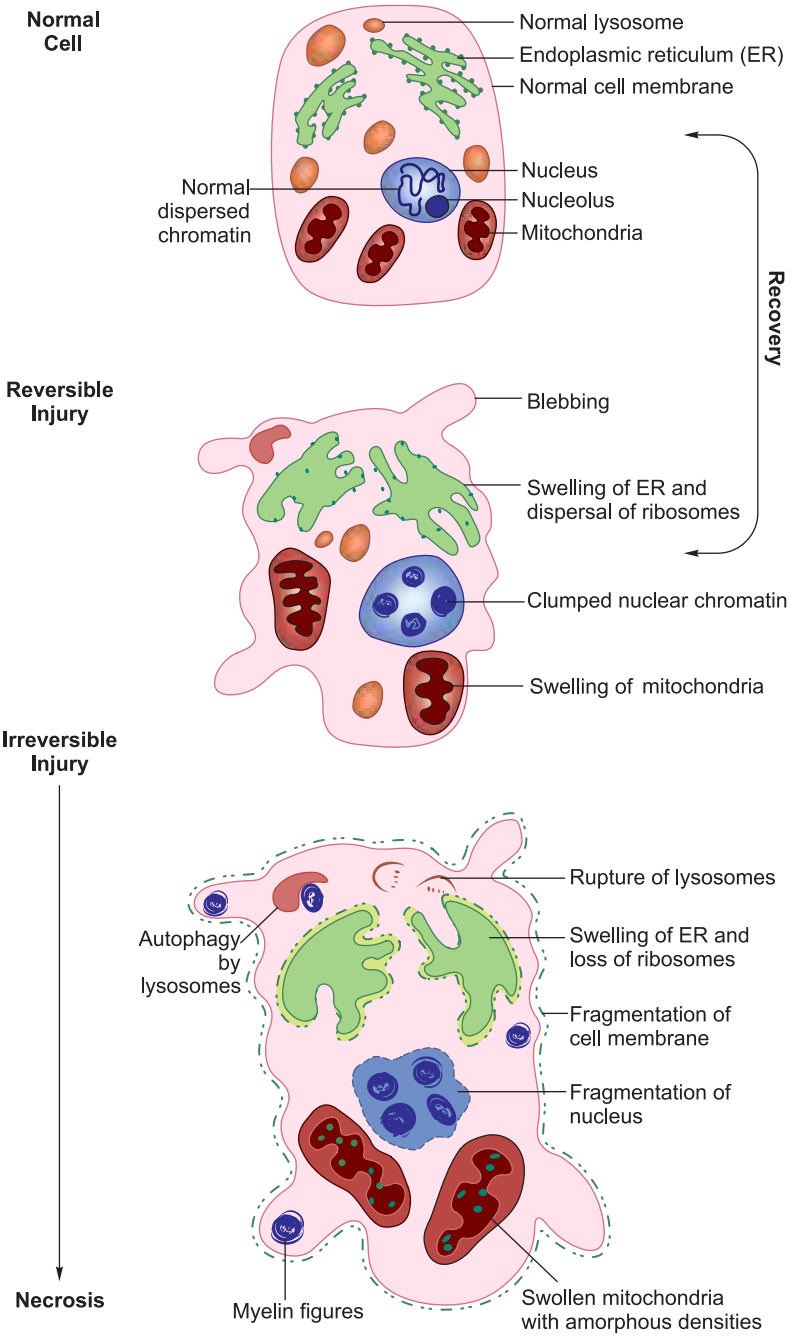


FIGURE 1.1. Cell injury.

Q. Enumerate and describe in brief different types of cellular adaptations.

Ans. Adaptive response may be in the form of

1. Hyperplasia
2. Hypertrophy
3. Atrophy
4. Metaplasia

Hyperplasia

Definition

Increase in number of cells in an organ or tissue leading to increased size/mass of the tissue or organ. *Hyperplasia takes place in cells, which are capable of synthesizing DNA*. In nondividing cells, only hypertrophy occurs.

Mechanism

- Production of transcription factors that induce genes encoding growth factors, receptors for growth factors and cell-cycle regulators.
- In *hormonal hyperplasia*, hormones themselves act as growth factors and trigger transcription of genes.
- In *compensatory hyperplasia*, there is proliferation of remaining cells and development of new cells from stem cells.

Types

1. Physiologic hyperplasia:
   (a) *Hormonal hyperplasia:* Hormonal stimulation increases the functional capacity of the tissue when needed, eg, breast and uterus in puberty, pregnancy and lactation.
   (b) *Compensatory hyperplasia:* Increase in tissue mass after damage or partial resection, eg, regeneration of liver after partial hepatectomy.
2. Pathologic hyperplasia: Hyperplasia due to excessive hormonal stimulation or excessive effects of growth factors on target cells, eg, *endometrial hyperplasia* (occurs when balance between progesterone and oestrogen is disturbed) and *benign nodular prostatic hyperplasia* or *NHP* (occurs due to androgen excess; Fig. 1.2).

Hypertrophy

Definition

Increase in size of the cell due to increased synthesis of structural components and not due to cellular swelling is known as hypertrophy. Nondividing cells, eg, myocardial fibres, undergo hypertrophy only. Dividing cells (stable cells, quiescent cells) undergo both hyperplasia and hypertrophy.

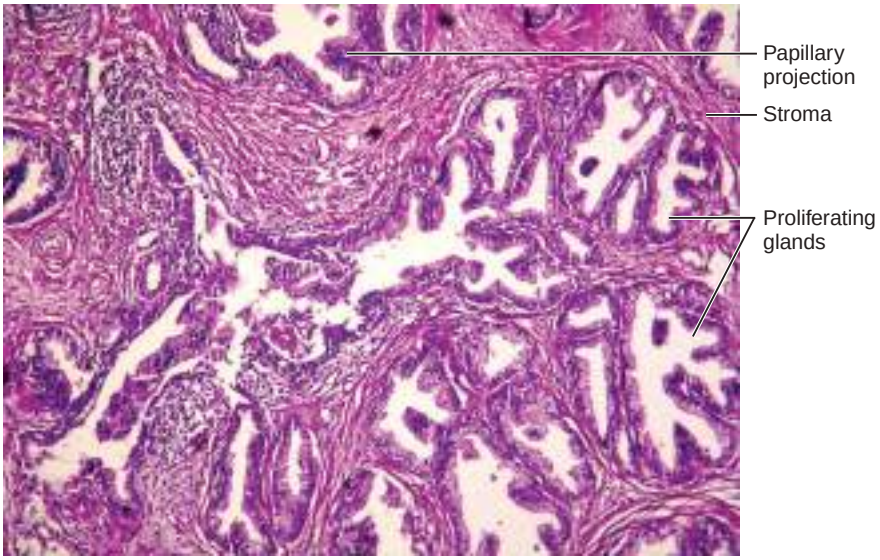


FIGURE 1.2. NHP prostate showing hyperplastic glands lying back to back. The glands are lined by two distinct layers of epithelium indicating benign nature of the lesion (H&E; 100×).

Mechanism

Induction of genes stimulates synthesis of cellular proteins, eg, genes encoding transcription factors, growth factors and vasoactive agents. In the heart, increased workload (mechanical stretch), growth factors (transforming growth factor-beta and Insulin-like growth factor-1) and α-adrenergic hormones activate signal transduction pathways (phosphoinositide-3-kinase/AKT pathway and downstream signalling of G-protein coupled receptors), which in turn activate transcription factors like GATA4 (critical transcription factor for proper mammalian cardiac development and essential for survival of the embryo), NFAT (nuclear factor of activated T cells) and MEF 2 (myocyte enhancer 2). They work together to increase synthesis of proteins responsible for cardiac hypertrophy.

Types

1. *Physiological hypertrophy*: This occurs due to increased functional demand and stimulation by growth factors and hormones, eg, uterine enlargement in pregnancy and breast hypertrophy during lactation.
2. *Pathological hypertrophy*:
   (a) Hypertrophy of cardiac muscle in systemic hypertension and aortic valve stenosis (chronic haemodynamic overload) leading to left ventricular hypertrophy (Fig. 1.3).
   (b) Compensatory hypertrophy, which occurs when an organ or tissue is called upon to do additional work or to perform the work of destroyed tissue or of a paired organ.

Atrophy

Definition

A decrease in size of a body organ, tissue or cell along with decreased function, owing to disease, injury or lack of use.

Mechanism

Atrophy is the result of decreased protein synthesis or increased protein degradation. Protein degradation is mediated by

- Lysosomal acid hydrolases, which degrade endocytosed proteins (taken up from extracellular environment, cell surface as well as some cellular components).
- Ubiquitin-proteasome pathway, which causes degradation of many cytosolic and nuclear proteins.

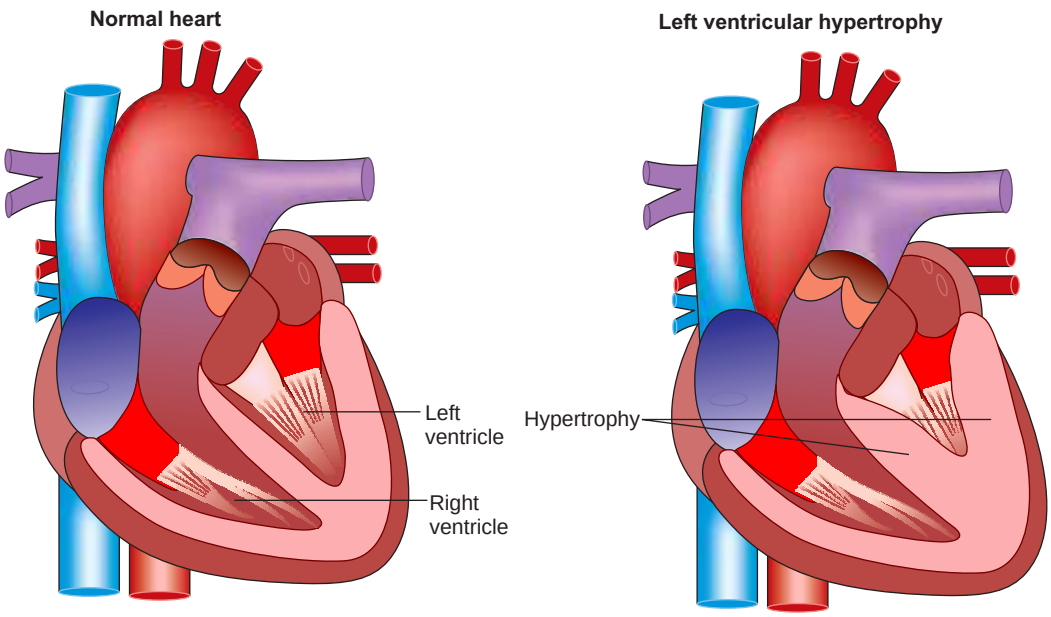


FIGURE 1.3. Pathological hypertrophy, left ventricle.

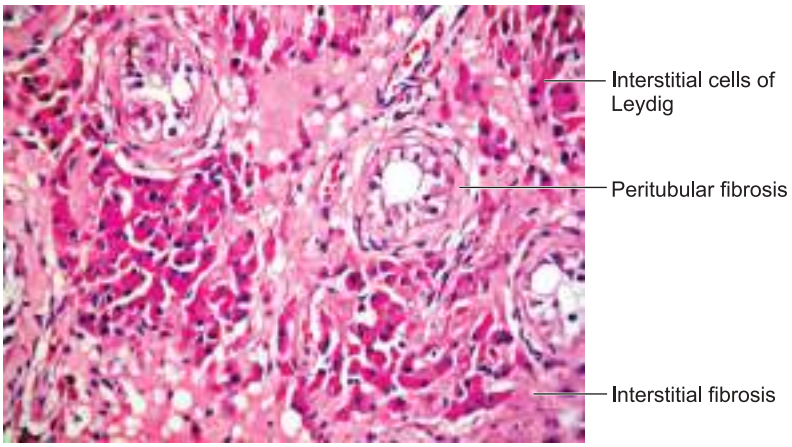


FIGURE 1.4. Atrophic testis showing marked loss of germ cells within the tubules, with peritubular and interstitial fibrosis and proliferation of interstitial cells of Leydig (H&E; 100×).

Types

1. *Physiological atrophy*: Common during early development, eg, atrophy of notochord or thyroglossal duct during fetal development and uterus after parturition.
2. *Pathological atrophy*:
   (a) Decreased workload due to immobilization and prolonged functional inactivity leads to disuse atrophy.
   (b) In denervation atrophy, there is loss of innervation of muscle which induces its wasting, as in polio and motor neuron disease.
   (c) Atherosclerosis can cause ischaemic atrophy.
   (d) Nutritional deficiency, eg, marasmus and cancer cachexia are associated with the use of skeletal muscle as a source of energy and lead to nutritional atrophy.
   (e) Loss of endocrine stimulation after menopause induces atrophy of reproductive organs.
   (f) Senile atrophy is an ageing-associated cell loss which is typically seen in tissues containing permanent cells, eg, brain and heart or testes (Fig. 1.4).

Metaplasia

Definition

Reversible change in which there is replacement of one adult/differentiated cell type (epithelial or mesenchymal) by another adult/differentiated cell type.

Mechanism

Occurs owing to altered/aberrant differentiation of stem cells due to their reprogramming.
Examples

- Columnar to squamous metaplasia in respiratory tract, in response to *chronic irritation* (cigarette smoking) and *vitamin-A deficiency*. Stones in excretory ducts of salivary glands, pancreas and gall bladder may also result in squamous metaplasia. Squamous metaplasia in cervix is usually associated with chronic infection (Fig. 1.5).
- Connective tissue metaplasia (formation of cartilage, bone or adipose tissue in tissues that normally do not contain these elements), eg, bone formation in muscle (myositis ossificans), which occurs after bone fracture.

Note: The factors that predispose to metaplasia, if persistent, may eventually lead to induction of cancer in metaplastic epithelium, eg, metaplasia from squamous to columnar epithelium in *Barrett's oesophagus may progress to adenocarcinoma oesophagus*.

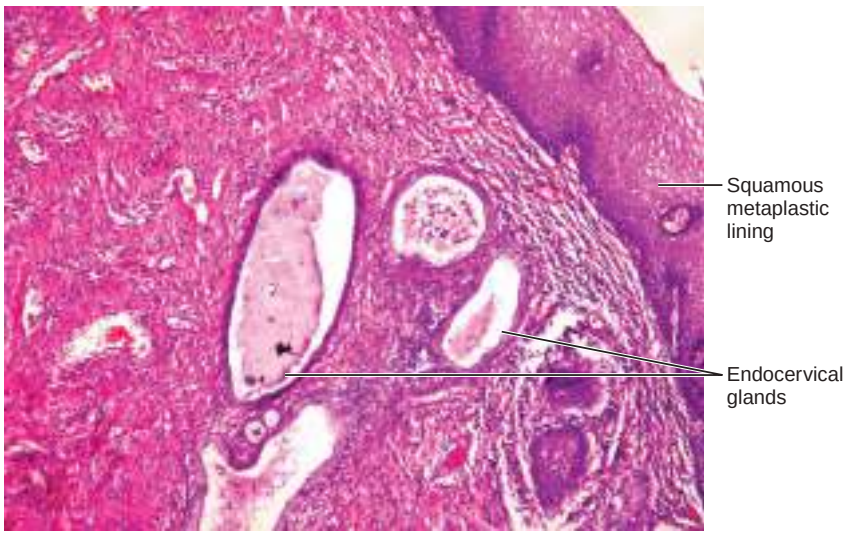


FIGURE 1.5. Section from cervix showing squamous metaplasia of the endocervical mucosa (H&E; 100×).

Q. Define dysplasia.

Ans. Dysplasia indicates disordered cellular development characterized by

- Loss of orientation of cells with respect to one another, eg, disorderly arrangement of the cells from basal to surface layer as in stratified squamous epithelium (*architectural disorientation*).
- Lack of uniformity of individual cells (*cellular pleomorphism*).
- Causes of dysplasia include diverse cellular insults, including physical, chemical and biological.
- It is typically seen in epithelial cells and may be reversible (at least in its early stage). More severe dysplasia is known to progress to carcinoma in situ and invasive carcinoma.
- Dysplastic cells are characterized by the following cellular features:
  - Accelerated cell proliferation (increased mitoses);
  - Nuclear abnormalities such as hyperchromasia (increased basophilia on staining with haematoxylin) and pleomorphism (altered nuclear size and nuclear shape; Fig. 1.6);
  - Increased nuclear-cytoplasmic ratio.

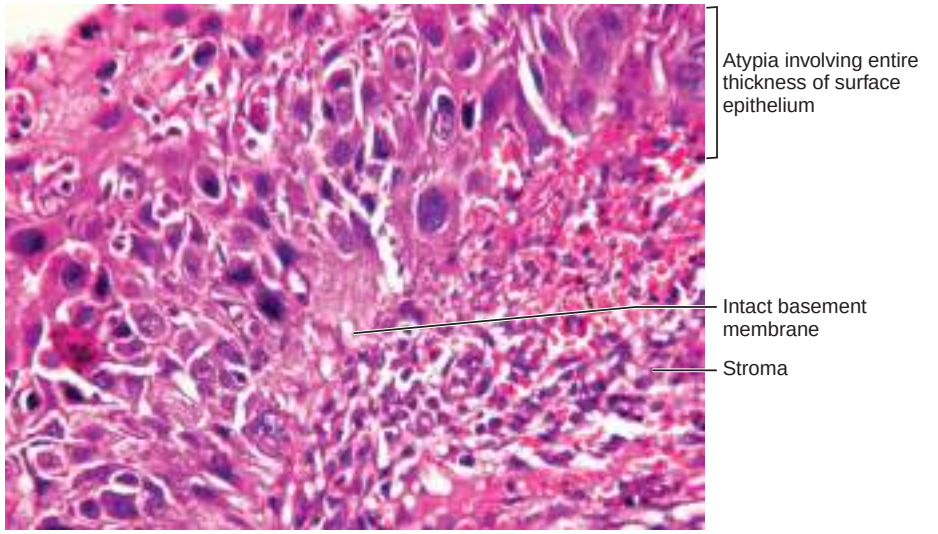


FIGURE 1.6. Stratified squamous epithelium showing severe dysplastic changes (diffuse atypia and loss of maturation) (H&E; 200×).

Q. Differentiate between metaplasia and dysplasia.

Ans. Differences between metaplasia and dysplasia are shown in Table 1.1.

TABLE 1.1. Differences between metaplasia and dysplasia

Features	Metaplasia	Dysplasia
Definition	Replacement of one adult epithelial or mesenchymal cell type by another	Disordered cellular development characterized by (a) Loss of orientation of cells with respect to one another (b) Lack of uniformity of individual cells
Types	Squamous, columnar (epithelial) and osseous, cartilaginous (mesenchymal)	Epithelial only
Cellular pleomorphism	Mature cellular development; no pleomorphism	Disordered cellular development due to aberrant/delayed maturation or differentiation; pleomorphism present
Natural history	Reversible on withdrawal of stimulus	May regress on withdrawal of inciting stimulus or progress to higher grades of dysplasia or carcinoma in situ

Q. Write briefly on aetiopathogenesis and biochemical basis of cell injury.

Ans. Sublethal or chronic injurious stimuli can cause 'reversible and irreversible cell injury'.

Causes of Cell Injury

- **Genetic**
  - Development defects (errors in morphogenesis)
  - Cytogenetic defects (chromosomal abnormalities)
  - Single gene defects (Mendelian disorders)
  - Multifactorial inheritance disorders
- **Acquired**
  - Hypoxia (ischaemia, anemia, carbon monoxide poisoning, cardiorespiratory failure).
  - Physical agents (trauma, thermal injury, radiation, electric shock, pressure changes)
  - Chemical agents/drugs (heavy metals, acids/alkalies, insecticides/herbicides, alcohol, smoking)
  - Microbial agents (bacteria, viruses, fungi, rickettsiae, parasites)
  - Immunological agents (autoimmunity, hypersensitivity)
  - Nutritional imbalance (deficiency of protein, calories, trace elements, vitamins, excess cholesterol).
  - Psychological factors

The cellular responses to pathological stimuli depend on

(a) Type, duration and severity of the injury.
(b) Type, status and adaptability of the target cell.

The most important *targets* of injurious stimuli are

(a) *Aerobic respiration* (involving mitochondrial oxidative phosphorylation and production of ATP)
(b) *Cell membrane*
(c) *Protein synthesis*
(d) *Cytoskeleton*
(e) *Genetic apparatus*

Biochemical Basis of Cell Injury

Cell injury occurs due to the following mechanisms:

- *ATP depletion*: ATP is required for
  - Membrane transport
  - Protein synthesis
  - Lipogenesis
  - Phospholipid turnover

  ATP depletion results in dysfunction in the above functions/mechanisms.
- *Damage due to oxygen and oxygen-derived free radicals* (See page 11)
- *Loss of calcium homeostasis:*
  - Normal cytosolic-free calcium levels are very low
  - Most intracellular calcium sequestered in endoplasmic reticulum and mitochondria
  - Injury causes influx of calcium across cell membrane and its release into cytosol from mitochondria and endoplasmic reticulum.
  - Increase in cytosolic calcium leads to activation of enzymes initiating cell injury

Q. Differentiate between reversible and irreversible cell injury.

Ans. Differences between reversible and irreversible cell injury are shown in Table 1.2.

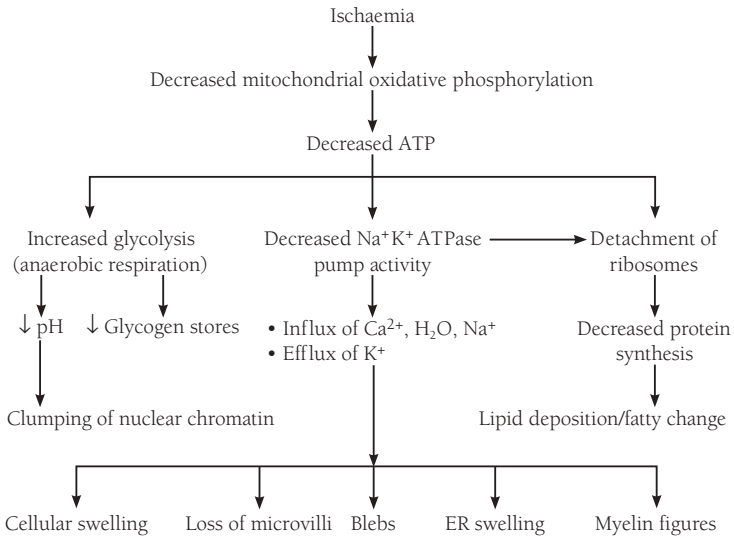
TABLE 1.2. Differences between reversible and irreversible cell injury

Features	Reversible injury	Irreversible injury
Definition	If the structural and functional changes, induced by an injurious stimulus, *can* revert to normal on removal of the same, it is called reversible injury	If the structural and functional changes, induced by an injurious stimulus, *cannot* be reversed even after removal of the same, it is called irreversible injury
Cell membrane		
(a) Blebbing, blunting, distortion	Present	Present; more prominent than reversible injury
(b) Defect	Absent	Present
Endoplasmic reticulum	Shows swelling only	Shows swelling and lysis
Ribosomes	Dispersed	Dispersed and destroyed
Lysosomes	Autophagy of organelles by lysosomes, no rupture	Rupture of lysosomes and autolysis of cell
Mitochondria	Swelling, small densities present	Swelling, large densities present
Nucleus	Clumping of nuclear chromatin	Pyknosis, karyolysis or karyorrhexis
Calcification	Absent	Dystrophic calcification may be seen

Q. Describe the sequence of events occurring in reversible and irreversible injury.

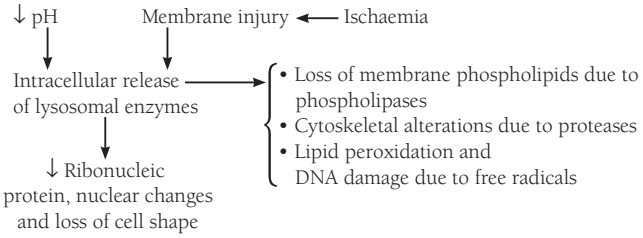
Ans. Sequence of events occurring in

- **Reversible injury** (Flowchart 1.4)



FLOWCHART 1.4. Sequence of events in reversible injury.

- **Irreversible injury** (Flowchart 1.5)



FLOWCHART 1.5. Sequence of events in irreversible injury.

Q. Write briefly on free radical-mediated cell injury.

Ans. Free radicals are chemical species with an unpaired electron in their outer orbit. They react with inorganic and organic molecules (proteins, lipids and carbohydrates), which are mainly present in membranes and nucleic acids.

Free radical production is induced by

- *Absorption of radiant energy*: UV rays, X-rays.
- *Enzymatic metabolism of exogenous chemicals/drugs*: CCl_4 to CCl_3.
- *Reduction–oxidation reaction processes that occur during normal metabolism*: Formation of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl ion ($\cdot OH$).
- *Reactions involving transition metals*: iron (Fenton reaction), copper, etc.
- *Reactions involving nitric oxide (NO):* acts as a free radical and can be converted to highly reactive peroxynitrite anion ($ONOO^-$) as well as NO_2 and NO_3^-.

Effects of free radicals:

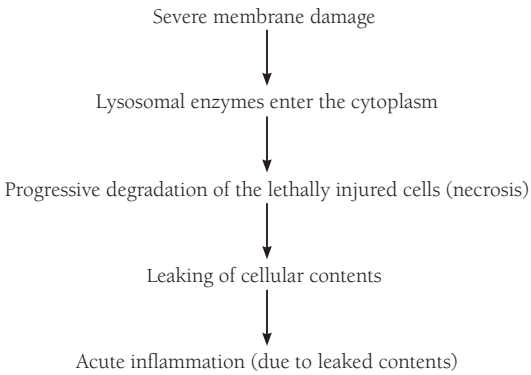
- *Lipid peroxidation*: Lipid and free radical interactions produce peroxides (*initiation*). Peroxides are reactive and unstable species, which start a chain reaction of lipid peroxidation (*propagation*). In some cases, chain reaction may be *terminated* by *antioxidants*.
- *Modification of proteins by oxidation*: Oxidation of amino acid residue side chain leads to formation of protein–protein cross-linkage and disruption of the protein backbone resulting in *protein fragmentation*.
- *DNA lesions*: Attack thymine and other nucleotides of nuclear and mitochondrial DNA to produce *single- or double-stranded breaks in DNA* as well as cross-linking of DNA strands.

Inactivation of free radicals is brought about by

- *Antioxidants*: vitamins A, C, E and β-carotene.
- *Iron- and copper-binding proteins*: transferrin, ferritin, lactoferrin, ceruloplasmin (decrease available free metal by binding to it).
- *Enzymes*: catalase, superoxide dismutase, glutathione peroxidase (catalyse free radical breakdown).

Q. Define necrosis and describe its various morphological patterns.

Ans. Disturbances of the external environment beyond the limits of homeostasis lead to premature cell death, which is called *necrosis*. Necrosis may be caused by ischaemia, infection, poisoning, etc., and is invariably pathological. It usually precipitates an inflammatory response and is accompanied by cell swelling, lysis and lysosomal leakage (Flowchart 1.6). Self-digestion of cells by enzymes liberated from its own lysosomes on the other hand is labelled *autolysis* (Table 1.3).



FLOWCHART 1.6. Sequence of events in cellular necrosis.

The morphological features of necrosis vary with its type. Changes common to most types include

1. **Cytoplasmic changes**
   - Increased eosinophilia of the cytoplasm, which is due to
     - loss of normal cytoplasmic basophilia caused by the loss of RNA and
     - denaturation of cytoplasmic proteins which then bind strongly to the dye eosin:
   - Glassy homogenous cytoplasm due to loss of glycogen.
   - Swelling and vacuolation of the cytoplasm (occurs after enzymatic digestion has started).
   - Cellular and organelle swelling may eventually lead to discontinuities in cell and organelle membranes and ultimately rupture.
   - Formation of myelin figures (phospholipid masses derived from damaged cell membranes).
2. **Nuclear changes**
   The changes in nucleus appear in one of the following three patterns:
   - Nuclear shrinkage and increased basophilia (pyknosis)
   - Nuclear fragmentation (karyorrhexis)
   - Loss or fading of basophilia due to DNase activity (karyolysis)

Morphological patterns of necrosis include

1. **Coagulative necrosis**
   - It is the most common pattern of necrosis and is caused by ischaemic injury resulting in hypoxic death of cells in all tissues except the brain.
   - There is preservation of the basic architectural outlines and type of tissue can be recognized but cellular details are lost.

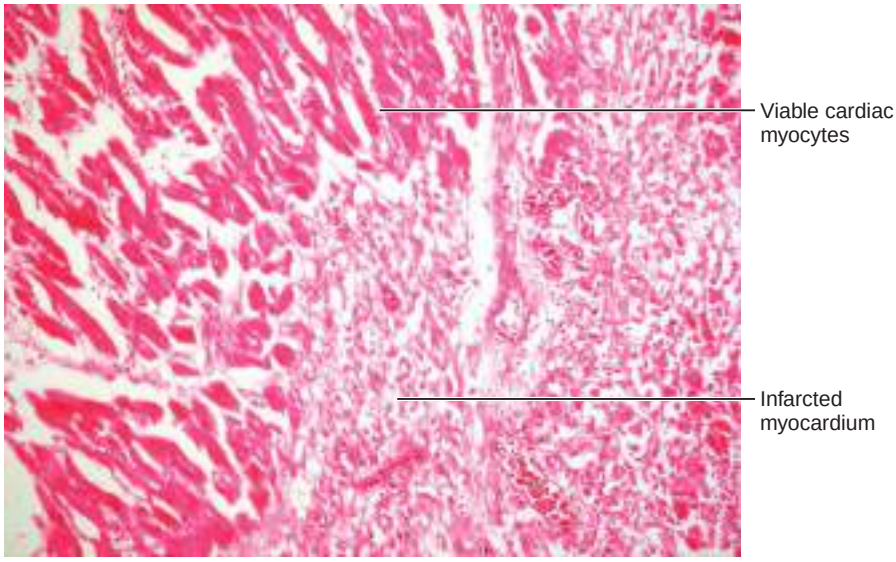
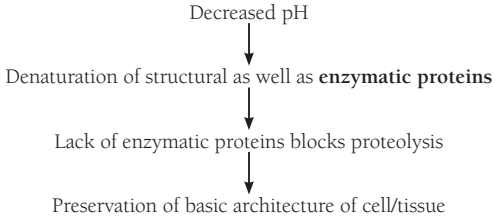


FIGURE 1.7. Infarcted myocardium surrounded by viable cardiac myocytes (H&E; 100×).

- Cell injury leads to increasing intracellular acidosis, which denatures not only structural proteins but also enzymatic proteins, and so blocks the proteolysis of the cell, thereby preventing loss of architecture of the tissue.
- On *gross examination*, the affected tissue is pale in colour and firm in texture.
- *Microscopically*, increased eosinophilia of the cytoplasm and decreased basophilia of the nucleus are observed. Myocardial infarction is an excellent example in which acidophilic, coagulated anucleate cells are seen (Fig. 1.7).

Mechanism of evolution of coagulative necrosis is shown in Flowchart 1.7.



FLOWCHART 1.7. Mechanism of evolution of coagulative necrosis.

2. **Liquefactive necrosis** (colliquative necrosis)
   - This occurs in situations in which enzymatic breakdown is more prominent than protein denaturation unlike coagulative necrosis (Table 1.4).
   - It is usually associated with bacterial or fungal infections because microbes stimulate the accumulation of leukocytes and liberation of enzymes from these cells.
   - The organ–cellular architecture is lost, and the tissue is digested and converted into a liquefied mass, which appears creamy yellow in colour and is called 'pus'.
   - Liquefactive necrosis is most commonly seen in organs that have a high-fat and low-protein content (eg, the brain), or those with a high-enzymatic content (eg, the pancreas), and typically causes gangrene of intestine (Fig. 1.8) and limbs and hypoxic death in brain.
   - Lack of a proper collagenous connective tissue framework in an organ also aids to this type of necrosis.

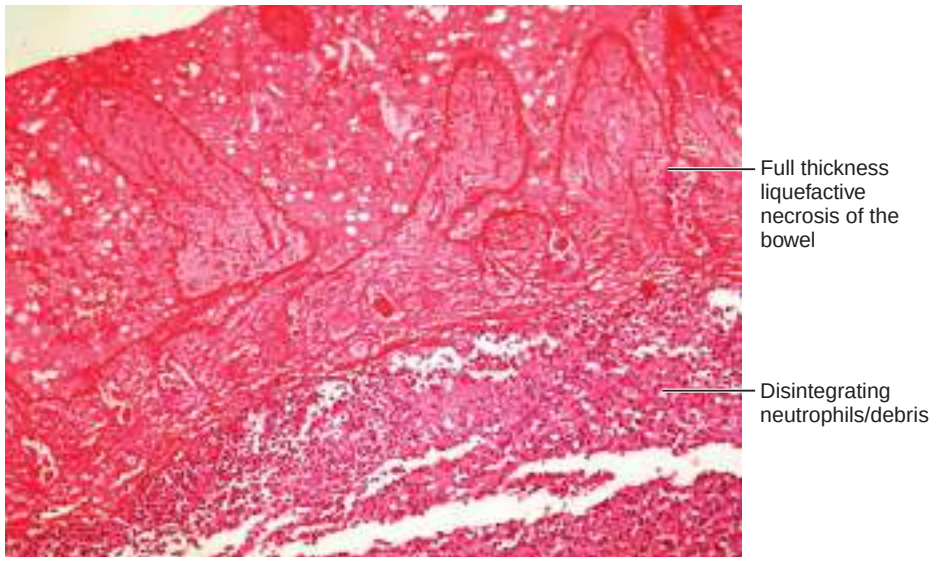


FIGURE 1.8. Liquefactive necrosis/gangrene of intestine (H&E; 100×).

Mechanism of evolution of liquefactive necrosis is shown in Flowchart 1.8.

Bacterial infection and accumulation of inflammatory cells
↓ *Release of enzymes*
Autolysis and heterolysis

FLOWCHART 1.8. Mechanism of evolution of liquefactive necrosis.

3. **Gangrenous necrosis**
   This is a clinical term, not a specific pattern of necrosis. It is usually used in context of the lower limbs, which have lost their blood supply and have undergone necrosis, initially coagulative (dry gangrene), and later liquefactive due to secondary bacterial infection and immigrating leukocytes (wet gangrene) (Table 1.6).

Mechanism of evolution of gangrenous necrosis is shown in Flowchart 1.9.

Bacterial infections and accumulation of inflammatory cells
↓ Release of enzymes
Autolysis and heterolysis

FLOWCHART 1.9. Mechanism of evolution of gangrenous necrosis.

4. **Caseous necrosis**
   - This type of necrosis is typically associated with tuberculous infection.
   - On gross examination, the necrotic areas appear cheesy white (caseous). Microscopically, the debris appears amorphous, eosinophilic and granular (Fig. 1.9), and is surrounded by a distinct inflammatory reaction called granulomatous reaction.
   - Tissue architecture is completely obliterated unlike coagulative necrosis (Table 1.5). Dystrophic calcification may be seen.
5. **Enzymatic fat necrosis**
   - It refers to a focal area of fat destruction that converts adipocytes to necrotic cells with shadowy outlines and basophilic calcium deposits, surrounded by an inflammatory reaction (Fig. 1.10).
   - It is typically seen in acute pancreatitis and traumatic fat necrosis of breast.

Mechanism of evolution of enzymatic fat necrosis in acute pancreatitis is shown in Flowchart 1.10.

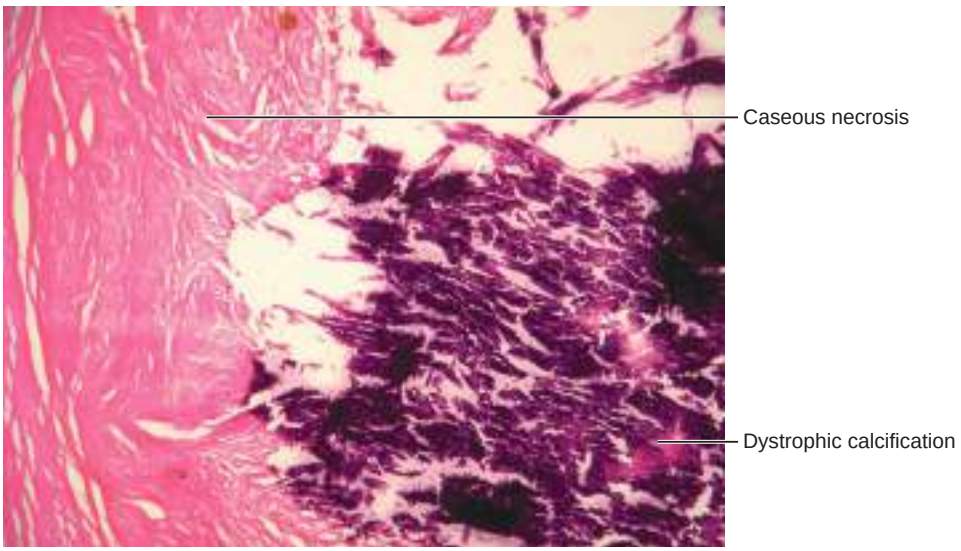


FIGURE 1.9. Section from a lymph node showing amorphous, eosinophilic and granular debris (caseous necrosis) surrounded by a granulomatous reaction composed of Langhans giant cells and chronic inflammatory cells (H&E; 100×).

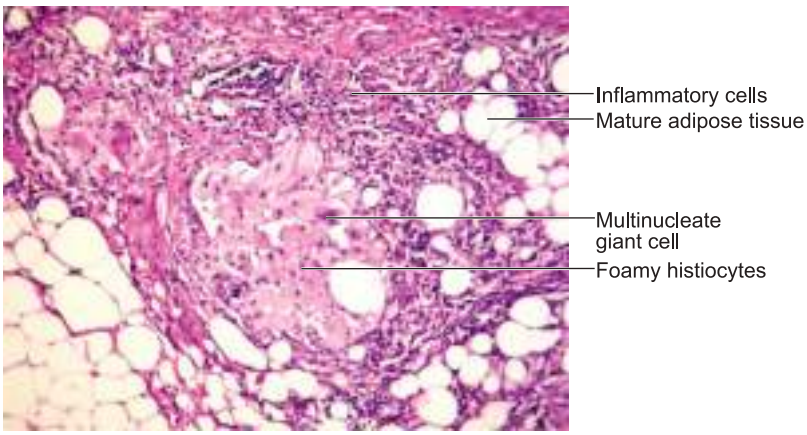


FIGURE 1.10. Fat necrosis in the breast showing disruption of normal adipocytes and accumulation of lipid-laden foamy histiocytes and a multinucleate giant cell (H&E; 200×).

Release of activated **pancreatic lipases** into pancreas and peritoneal cavity

↓

Focal areas of destruction of fat and release of fatty acids

↓

Released fatty acids combine with calcium **(saponification)**

↓

Chalky white areas

FLOWCHART 1.10. Mechanism of evolution of enzymatic fat necrosis.

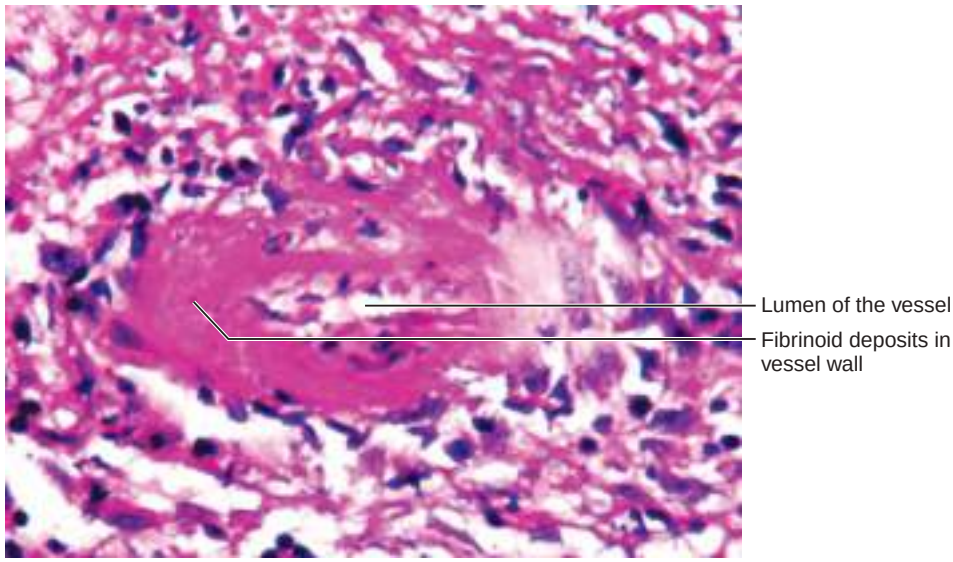


FIGURE 1.11. Fibrinoid necrosis of vessel wall seen as bright pink smudgy deposits (H&E; 200×).

6. **Fibrinoid necrosis**
   - Deposition of bright, smudgy, eosinophilic fibrin-like material in vessel wall (Fig. 1.11).
   - The fibrinoid material is composed of degenerated collagen and ground substance.
   - It is usually seen in patients with malignant hypertension and immunological injury (vasculitis—polyarteritis nodosa). It may also be seen in rheumatic fever, rheumatoid arthritis, hepatitis B virus (HBV) infection, systemic lupus erythematosus (SLE), etc.

Q. Differentiate between autolysis and necrosis.

Ans. Differences between autolysis and necrosis are shown in Table 1.3.

TABLE 1.3. Differences between autolysis and necrosis

Features	Autolysis	Necrosis
Definition	Self-digestion of cells by enzymes liberated from its own lysosomes	Spectrum of morphologic changes that follow cell death in living tissue, resulting from the progressive degradative action of enzymes on lethally injured cells
Reaction	• In *living tissue*, inflammatory cells may be present • In *post-mortem* cases, there is complete absence of inflammatory cells	• Presence of inflammatory cells • Does not occur post-mortem
Calcification	Absent	Dystrophic calcification may be present

Q. Differentiate between coagulative and liquefactive necrosis.

Ans. Differences between coagulative and liquefactive necrosis are shown in Table 1.4.

TABLE 1.4. Differences between coagulative and liquefactive necrosis

Features	Coagulative necrosis	Liquefactive necrosis
Cause	Hypoxic/ischaemic injury in all tissues except in brain, eg, myocardial, renal or placental infarction	• Bacterial and fungal infections • Hypoxic injury in brain
Tissue architecture	Tissue architecture is preserved; the basic outline of cell is intact, although cytoplasmic and nuclear details are lost	Both cell outline and intracellular details are lost; tissue architecture is not preserved
Pathogenesis	Due to intracellular acidosis, structural as well as enzymatic proteins are denatured and proteolysis is blocked; dead cells are removed by fragmentation and phagocytosis	Hydrolytic enzymes from bacteria and fungi as well as inflammatory cells cause complete digestion of dead cells and formation of pus (lysis)
Morphology	Conversion of cells into acidophilic, coagulated, anucleate units	No cellular outline/tissue architecture recognized

Q. Differentiate between coagulative and caseous necrosis.

Ans. Differences between coagulative and caseous necrosis are shown in Table 1.5.

TABLE 1.5. Differences between coagulative and caseous necrosis

Features	Coagulative necrosis	Caseous necrosis
Cause	Hypoxia	Tuberculous infection of lymph nodes, lungs, skin, etc.
Pathogenesis	Due to intracellular acidosis, structural as well as enzymatic proteins are denatured and proteolysis is blocked	Delayed hypersensitivity reaction to *mycobacterial* capsular antigens
Gross	Affected tissue is firm in texture	Cheesy white appearance
Tissue architecture	Preserved	Completely obliterated

Q. Differentiate between dry and wet gangrene.

Ans. Differences between dry and wet gangrene are shown in Table 1.6.

TABLE 1.6. Differences between dry and wet gangrene

Features	Dry gangrene	Wet gangrene
Cause	Mainly arterial occlusion (coagulative necrosis)	More in venous occlusion; obstruction invariably followed by secondary bacterial infection (liquefactive necrosis)
Distribution	Limbs	More common in bowel
Gross appearance	Organ is dry, shrunken and black	Moist, soft, swollen
Line of demarcation	Present at junction between healthy and gangrenous parts	Not clear
Putrefaction	Limited (no infection and less blood supply)	Marked
Presence of bacteria	Absent, little or no septicaemia	Overwhelming septicaemia present
Prognosis	Better	Poor

Q. Define apoptosis and describe its morphology, biochemical basis and underlying mechanism.

Ans. Apoptosis is a form of genetically programmed cell death designed to eliminate unwanted host cells through activation of a coordinated series of events. It occurs in physiological and pathological conditions, in contrast with necrosis, which is always pathological (Table 1.7).

- **Physiological apoptosis:**
  - During development/embryogenesis (implantation and organogenesis)
  - Hormone-dependent involution (regression of lactational changes in breast and prostatic atrophy)
  - Cell deletion in proliferating cell population such as intestinal crypt epithelia
  - Apoptosis of immune T and B cells as in clonal deletion or cell death induced by cytotoxic T cells
  - Cell ageing
- **Pathological apoptosis:**
  - Cellular damage by diseases/noxious agents, eg, councilman bodies in hepatitis
  - Pathological atrophy in parenchymal organs after duct obstruction, eg, salivary gland and pancreas
  - Pathological atrophy in hormone-dependent organs, eg, prostate
  - Cell death in the tumours
  - Low doses of thermal injury, radiation and anticancer drugs

Sequence of Morphological Changes in Apoptosis (Fig. 1.12)

1. Cell shrinkage (increased density of the cytoplasm with tightly packed organelles)
2. Chromatin condensation under the nuclear membrane followed by nuclear fragmentation
3. Formation of cytoplasmic blebs followed by fragmentation into apoptotic bodies (surface blebbing followed by fragmentation into membrane-bound apoptotic bodies)
4. Phagocytosis of apoptotic bodies (ingestion by macrophages followed by lysosomal degradation)

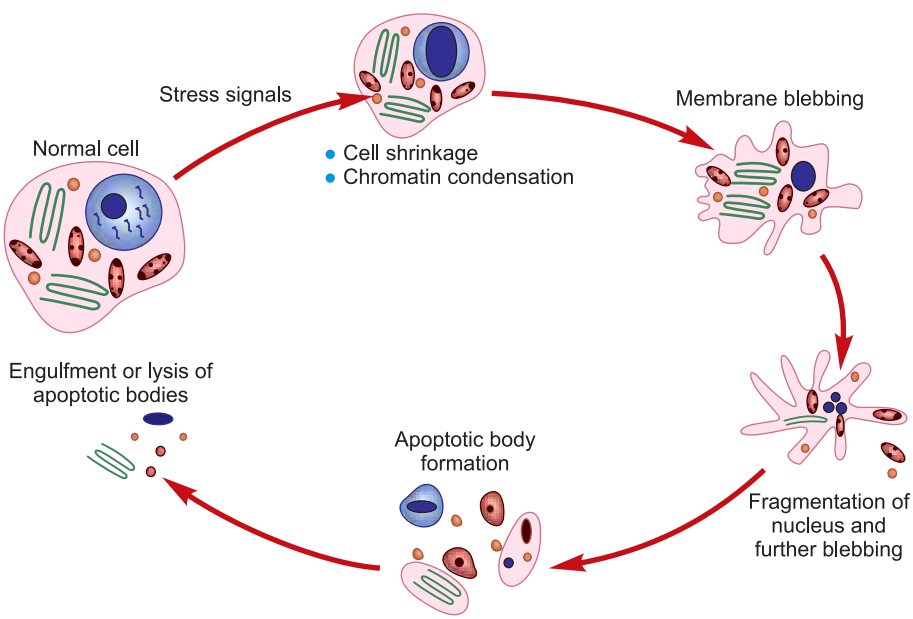


FIGURE 1.12. Sequence of morphological changes in apoptosis.

Sequence of Biochemical Events in Apoptosis

1. **Protein cleavage by proteolytic enzymes**

   Activation of caspases (family of cysteine proteases having a unique ability to cleave after aspartic acid residues)

   ↓

   Protein hydrolysis → Cleavage or breakup of nuclear scaffold
   Protein hydrolysis → Cleavage or breakup of cytoskeletal proteins

2. **Protein cross-linkage**

   Activation of transglutaminases

   ↓

   Cross-linking of cytoplasmic proteins leading to covalently linked shrunken cells

   ↓

   Easy breakdown into apoptotic bodies

3. **DNA condensation and breakdown**

   DNA breakdown into large pieces (50–300 kb)

   ↓

   Internucleosomal cleavage by endonucleases forming oligonucleosomes (180–200 bp) visualized on agarose gel electrophoresis as DNA ladders

4. **Recognition of dying cells by phagocytes**

   Flip-flop of apoptotic cell

   ↓

   Phosphatidylserine and thrombospondin flip on the external surface from the inner layers

   ↓

   Easy recognition and phagocytosis of the apoptotic cell

Mechanism of Apoptosis

Apoptosis is the end point of an energy-dependent cascade of molecular events having four steps.

1. **Initiation of apoptosis by activation of signalling pathways:**
   There are two main signalling pathways in apoptosis.
   (a) Extrinsic/death receptor-initiated pathway (Flowchart 1.11): involves extracellular or transmembrane signals, which may be *positive* (leading to initiation) or *negative* (opposing initiation). Extrinsic pathway is mainly initiated by engagement of plasma membrane death receptors on cells. Death receptors are members of the tumour necrosis factor (TNF)-receptor family that contains a cytoplasmic domain called death domain because it delivers signals for apoptosis. Important death receptors include TNFR1 and a related protein called Fas (also called CD 95; Flowchart 1.11). The ligand for Fas is Fas ligand (Fas L) which is expressed on T cells.

Extracellular signals
- *Injuries*: radiation, toxins and free radicals
- *Withdrawal of growth factors, hormones or cytokines*
- *Receptor–ligand interactions* (Fas–Fas ligand, TNF–TNF receptor)

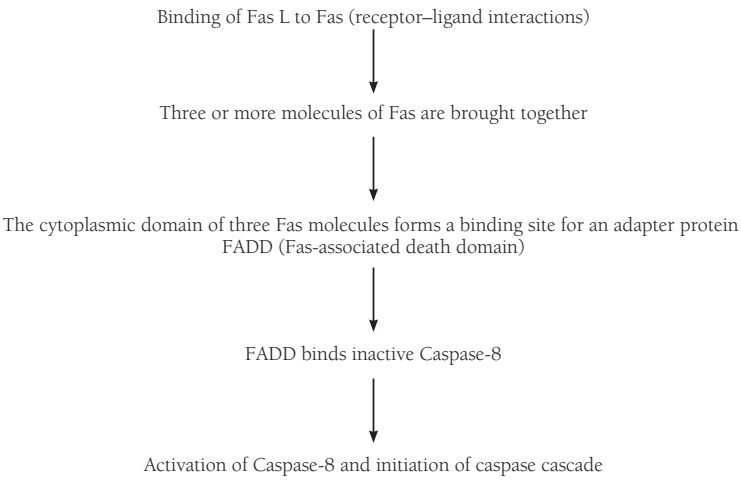
↓

Act on intracellular regulatory molecules

OR

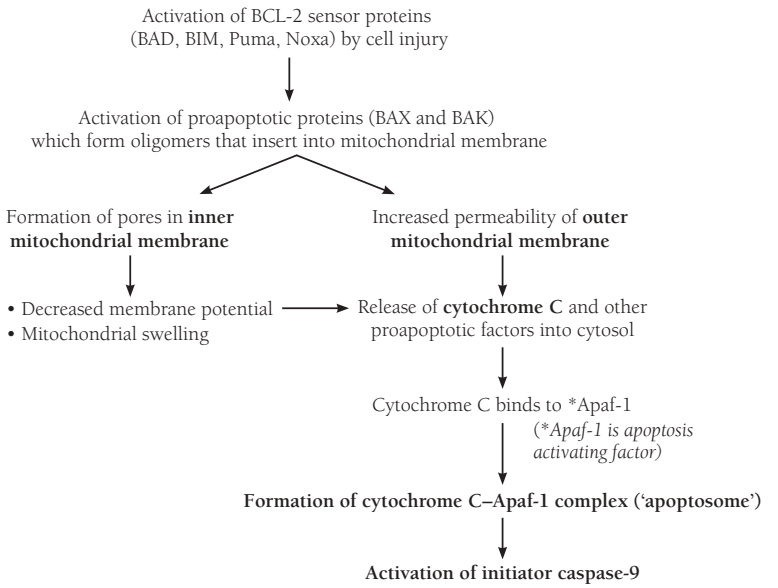
Directly affect targets within the cell (eg, physicochemical agents like heat, radiation, viruses and xenobiotics and glucocorticoids directly bind to nuclear receptors)

FLOWCHART 1.11. Extrinsic/death receptor-initiated pathway.



FLOWCHART 1.11. cont'd

(b) Intrinsic/mitochondrial pathway (the major mechanism of apoptosis; Flowchart 1.12):



FLOWCHART 1.12. Intrinsic/mitochondrial pathway.

2. **Control and integration:** Commitment or abortion of lethal signals is controlled byBCL2 family of proteins which include 'antiapoptotic proteins' (BCL2, BCLXL and MCL1); 'proapoptotic proteins' (BAX and BAK); and 'BCL2 sensor proteins' (BAD, BIM, Puma, Noxa). Also, the cytoplasm of normal cells contains inhibitors of apoptosis (IAP) which are neutralized by proapoptotic factors.
3. **Execution phase:** Proteolytic cascade involving *execution caspases* (caspases 3 and 6). *Caspase 3* also converts a cytoplasmic DNase into an active form by cleaving the inhibitor of this enzyme (this DNase induces internucleosomal cleavage of DNA).
4. **Removal of dead cells:** Early recognition and removal by macrophages. Removal is aided by
   (a) Expression of phosphatidylserine: in normal cells phosphatidylserine is present in the inner leaflet of the plasma membrane; during apoptosis there is turning out of

the phosphatidylserine so that it is expressed on the outer membrane and is easily recognized by the macrophage.
(b) Secretion of soluble factors by apoptotic cells, eg, thrombospondin, which recruit macrophages.
(c) Coating of apoptotic cells by natural antibodies and proteins of the complement system, which are easily recognized by macrophage receptors.

Q. Differentiate between apoptosis and necrosis.

Ans. Differences between apoptosis and necrosis are shown in Table 1.7.

TABLE 1.7. Differences between apoptosis and necrosis

Features	Apoptosis	Necrosis
Definition	Programmed and coordinated cell death, which eliminates unwanted/harmful cells or removes cells damaged beyond repair	Spectrum of morphologic changes that follow cell death in living tissue, largely resulting from the progressive degradative action of enzymes on lethally injured cells
Causes	May be physiological or pathological	Always pathological, eg, hypoxia, toxins
Involves	Single or small groups of cells	Large groups of cells
Inflammation	Absent	Present
Cellular change	Cell shrinkage	Cell swelling
Cell membrane	Bleb formation	Membrane disruption
Nucleus	Chromatin condensation followed by fragmentation	Nuclear pyknosis, karyolysis and karyorrhexis
Removal of cell	Phagocytosis of apoptotic bodies by macrophages	Enzymatic digestion or phagocytosis of cell debris by macrophages
Lysosomes/other organelles	Intact	Hydrolytic enzyme release due to rupture
Mechanism	Genetically coordinated	Due to ATP depletion, free radicals, mitochondrial damage, etc.
Agarose gel electrophoresis	Stepladder DNA pattern	Diffuse DNA pattern

Q. Enumerate the disorders associated with apoptosis.

Ans.

1. Disorders associated with decreased apoptosis: cancer, autoimmunity
2. Disorders associated with increased apoptosis:
   (a) Neurodegenerative diseases (Alzheimer, Huntington, Parkinson)
   (b) Ischaemic injury in stroke and myocardial infarction
   (c) Death of virus-infected cells as in AIDS

Q. Enumerate the steps in diagnosis of apoptosis.

Ans. Diagnosis of apoptosis:

1. Stepladder pattern on agarose gel electrophoresis
2. Terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling (TUNEL) technique for in vivo detection
3. H&E, Feulgen and acridine orange staining of apoptotic cells
4. Measurement of cytosolic cytochrome c and activated caspase
5. Expression of phosphatidylserine on the outer leaflet of the plasma membrane by apoptotic cells enables their recognition by using the dye Annexin V

Q. Classify intracellular accumulations and write briefly about them.

Ans. Intracellular accumulations include the following:

Accumulation of Normal Cellular Constituent in Excess

1. **Water**
   (a) **Cloudy swelling**
      (i) A form of reversible injury, cloudy swelling is also called granular degeneration (named so because of the presence of prominent protein granules in the cytoplasm)
      (ii) It commonly affects hepatocytes, renal tubular cells (Fig. 1.13) and myocardium
      Gross pathology: The affected organ is enlarged, soft and pale (pallor is due to mechanical compression of capillaries by retained water).
      Microscopy: Cells are swollen, full of proteinaceous granules (thought to be fragmented mitochondrial proteins or products of disturbed protein metabolism), and have frayed cell margins. Nuclei are normal in early stages, but could later appear faint or intensely staining.
   (b) **Hydropic/vacuolar degeneration**
      (i) This is an extension of changes seen in cloudy swelling
      (ii) Affected cells are ballooned, pale, watery and vacuolated
      (iii) Vacuoles coalesce and push nucleus towards the periphery
      (iv) Cell bursts and nucleus undergoes karyorrhexis/lysis
2. **Fat:** Abnormal accumulation of triglycerides in the cytosol of parenchymal cells is called fatty change (steatosis). It mainly affects liver and heart but can also be seen in muscle and kidney.

   Causes of fatty liver
   - Alcohol abuse.
   - Starvation/malnutrition.
   - Diabetes mellitus.
   - Obesity.
   - Hepatotoxins like CCl_4, ether, aflatoxins.
   - Certain drugs, like steroids, tetracycline and aspirin (Reye syndrome).
   - Hypoxia in anaemia and cardiac failure.
   - Late pregnancy.
   - Chronic illness, like tuberculosis.

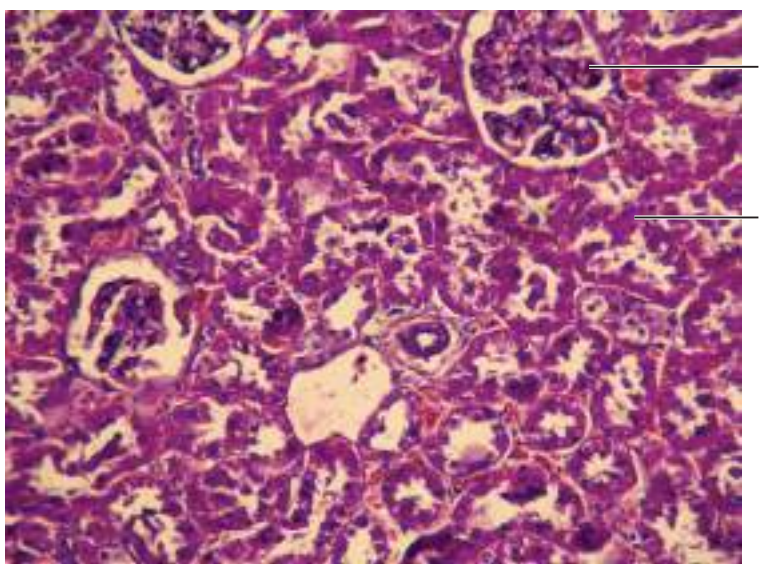
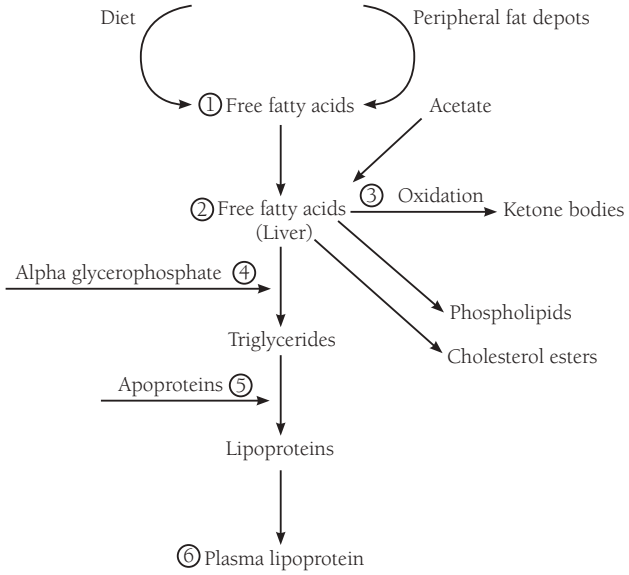


FIGURE 1.13. Section from cloudy swelling kidney showing tubules lined by swollen cells with frayed margins and increased granularity (H&E; 200×).

Mechanism of development of fatty liver is shown in Flowchart 1.13.



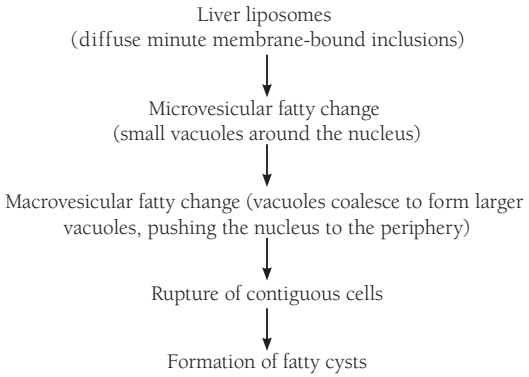
FLOWCHART 1.13. Mechanism of development of fatty liver.
1. Excessive entry of free fatty acids into the liver (starvation, toxins, diabetes mellitus, anoxia).
2. Increased synthesis of free fatty acids in the liver (obesity, alcohol abuse).
3. Decreased oxidation of fatty acids into ketones (anoxia, starvation).
4. Increased esterification of fatty acids into triglycerides (alcohol).
5. Decreased synthesis of apoproteins (CCL4 toxicity, protein energy malnutrition).
6. Defective excretion of lipoproteins.

Morphological features associated with fatty change

(a) **Liver**

Gross pathology: In diffuse fatty change the organ appears enlarged, pale, soft, yellow and greasy. Focal fatty change is seen as yellow mottling.

Microscopy (Fig. 1.14; Flowchart 1.14)



FLOWCHART 1.14. Sequence of events in the evolution of fatty liver.

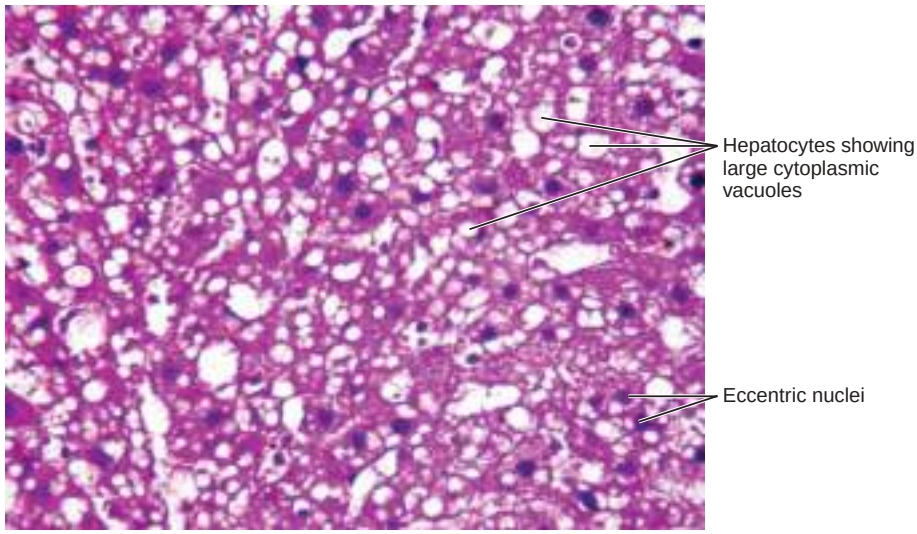


FIGURE 1.14. Hepatocytes showing fatty change. The fat vacuole has pushed the nucleus into the peripherally displaced cytoplasm (H&E; 200×).

(b) **Heart**
Lipid may be found in the myocardium as small droplets. Two patterns are observed depending on the type of hypoxic stimulus:
(i) *Prolonged moderate hypoxia:* causes focal intracellular deposits of fat that create grossly yellow bands of myocardium alternating with darker red-brown normal myocardium (*tigered effect*).
(ii) *Profound hypoxia or myocarditis:* affects myocytes uniformly.

3. **Carbohydrates:** Accumulation of carbohydrates is seen in conditions such as glycogenosis and mucinous degeneration.
4. **Proteins:**
Proteins can accumulate as
(a) Colloid droplets (reabsorption droplets in proximal renal tubules seen in renal diseases associated with excessive protein loss in the urine).
(b) Russell bodies (active synthesis of immunoglobulins leads to excessive amounts of secretory protein in plasma cells causing huge distension of endoplasmic reticulum, which appear as large eosinophilic inclusions).
(c) Defective secretion and transport of proteins, as in α-1 antitrypsin deficiency, results in accumulation of misfolded protein in ER causing ER stress as well as loss of protein function inducing emphysema and cirrhosis.

Accumulation of Abnormal Cellular Constituents

Hyaline Change (Derived From Hyalos Glass)

It is defined as deposition of a glassy, homogenous, eosinophilic material resulting from a variety of heterogeneous pathologic conditions. Hyaline may be

(a) Intracellular: when it is seen within epithelial cells, eg,
(i) Hyaline droplets: Observed in proximal convoluted tubules due to excessive reabsorption of plasma proteins.
(ii) Hyaline degeneration: Hyaline deposits in voluntary muscle, eg, degeneration of rectus abdominis.
(iii) Mallory's hyaline: Aggregates of intermediate filaments seen in hepatocytes in alcoholic injury.
(iv) Hyaline inclusions: Nuclear and cytoplasmic inclusions seen in viral infections.
(v) Russell bodies: Excessive immunoglobulins in endoplasmic reticulum of plasma cells.

(b) Extracellular: Seen in connective tissue, eg, hyaline degeneration in leiomyomas (Fig. 1.15), hyaline arteriosclerosis and hyalinization of glomeruli in chronic glomerulonephritis.

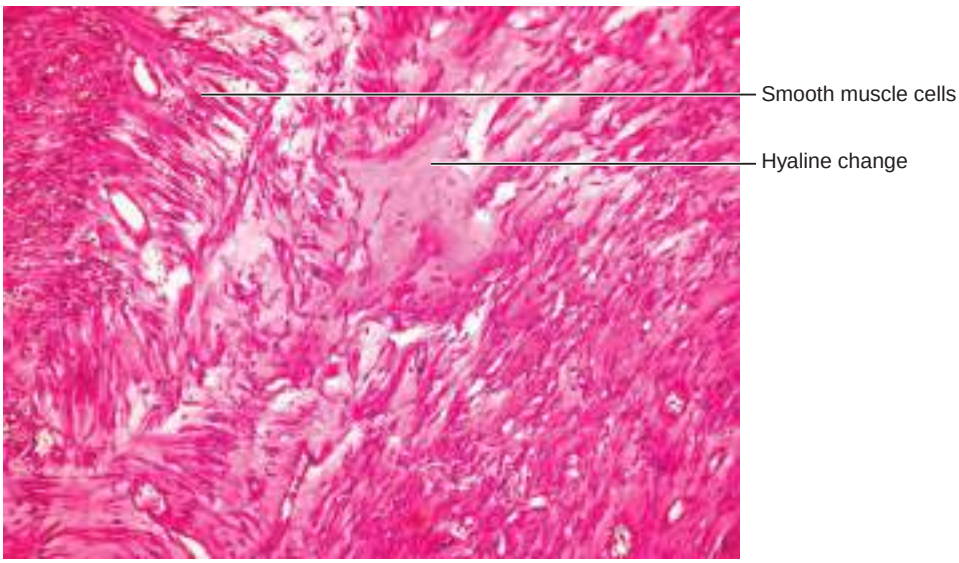


FIGURE 1.15. Hyaline degeneration in a leiomyoma (H&E; 200×).

Accumulation of Pigments

Pigment refers to material that has colour and can be seen without staining. In pathology, pigments play an important role in the diagnosis of diseases such as gout, jaundice, melanomas, albinism and haemorrhage. They can be classified as

1. **Endogenous pigments**
   (a) **Melanin:**
      (i) Nonhaemoglobin-derived brown-black pigment (Fig. 1.16)
      (ii) Normally present in skin, hair, choroids, meninges and adrenal medulla
      (iii) Synthesized by melanocytes and dendritic cells

      Disorders of pigmentation involving melanin:
      - Hyperpigmentation:
        - Addison disease
        - Adrenogenital syndrome
        - Chloasma/melasma

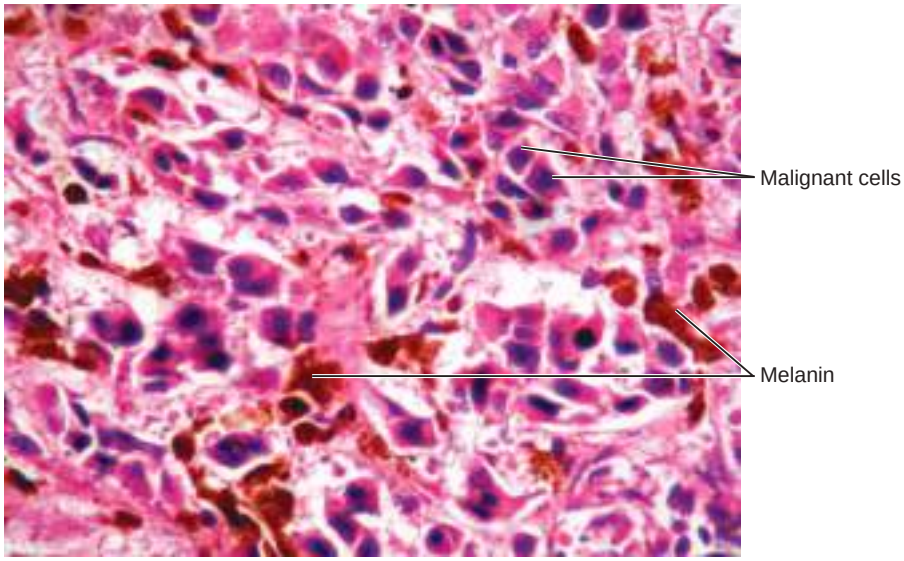


FIGURE 1.16. Intracellular and extracellular melanin deposits in a malignant melanoma (H&E; 400×).

- Chronic arsenical poisoning (raindrop pigmentation)
- Linea nigra (a hyperpigmented line found on the abdomen during pregnancy)
- Café-au-lait spots (neurofibromatosis, Albright syndrome)
- Perioral pigmentation in Peutz–Jeghers syndrome
- Melanocytic tumours/nevi
- Dermatopathic lymphadenitis

- Hypopigmentation:
  - Albinism
  - Vitiligo
  - Leprosy
  - Post-inflammatory scarring
  - Radiation dermatitis

Staining characteristics of melanin:

Can be bleached with hydrogen peroxide and stained with *Masson–Fontana argentaffin stain;* this forms the basis of differentiation of melanin from melanin lookalikes, eg, homogentisic acid seen in alkaptonuria and carbon seen in anthracosis.

(b) **Lipofuscin**

(i) Lipid-derived wear and tear pigment (associated with atrophied cells of old age and wasting)
(ii) Derived from the Latin word 'fuscus', meaning brown
(iii) Sometimes called 'residual bodies' (collection of indigestible material in the lysosomes after intracellular lipid peroxidation)
(iv) Yellow-brown, granular, intracytoplasmic (perinuclear in location)
(v) Seen in *myocardium*, *hepatocytes*, *Leydig cells* and *neurons*

Staining characteristics of lipofuscin:

- *Acid fast* (AFB positive)
- *Autofluorescent*
- Stains positive with *fat stains*
- Reduces ferricyanide to ferrocyanide (*Schmorl reaction*)

(c) **Haemosiderin**

(i) Golden-yellow to brown, crystalline granular pigment, which stains with Prussian blue stain (Fig. 1.17).
(ii) *Haemosiderosis* is defined as the presence of stainable iron in tissue. Based on distribution, it may be classified as

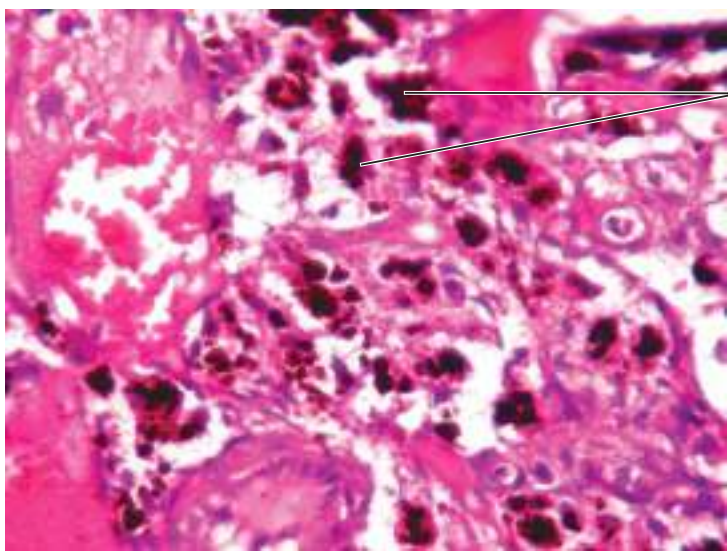


FIGURE 1.17. Haemosiderin deposits in the lining of a bone cyst (H&E; 400×).

FIGURE 1.18. Bile pigment in a section from liver (H&E; 200×).

***Localized*:** deposits in macrophages, fibroblasts, endothelial and alveolar cells secondary to haemorrhage in tissues, eg, bruise, black eye, brown induration of lung and infarction.

***Generalized*:** deposits in reticuloendothelial cells (liver, spleen, bone marrow) or parenchymal organs (liver, pancreas, kidney, heart) secondary to haemolytic disorders, blood transfusions, parenteral iron therapy, idiopathic haemosiderosis and Bantu disease.

(iii) Severe progressive iron overload leading to fibrosis and organ failure is called *haemochromatosis*.

(d) **Acid haematin** (**haemozoin**)
- (i) Haemoprotein-derived brown-black pigment seen in malaria.
- (ii) Does not stain with Prussian blue (because iron is in ferric form).

(e) **Bilirubin**
- (i) Major pigment found in bile (Fig. 1.18), stains with *Gmelin reaction* (oxidation by concentrated nitric acid to red/blue-green products) and *Stein's technique* (oxidation by iodine to form a green biliverdin pigment).
- (ii) Derived from haemoglobin but contains no iron.
- (iii) Excess of this pigment in tissues causes *jaundice*.

2. **Exogenous pigments**

(a) Inhaled pigments: The most common inhaled pigment is carbon; others include silica, iron and asbestos. Inhaled carbon is taken up by alveolar macrophages and may settle in the lungs or may be carried by lymphatics to hilar lymph nodes.

(b) Ingested pigments: Chronic ingestion of metals can cause the following conditions:
- (i) ***Argyria*:** Due to chronic ingestion of silver; causes brownish pigmentation of skin, bowel and kidney.
- (ii) ***Chronic lead poisoning*:** Blue pigmentation on teeth at gum line is a feature of chronic lead poisoning.
- (iii) ***Melanosis coli*:** Pigmentation of colon associated with prolonged ingestion of cathartics.
- (iv) ***Carotenaemia*:** Yellow-red discoloration of skin caused by ingestion of carrots.

(c) Injected pigments: These include India ink, cinnabar, carbon, etc., used in tattooing.

Q. Write briefly on pathologic calcification.

Ans. Pathologic calcification is defined as abnormal deposition of calcium salts together with smaller amounts of iron, magnesium and mineral salt forms.

Types of Pathological Calcification (Table 1.8)

1. **Dystrophic calcification:** Deposition of calcium in dead tissue, eg, areas of necrosis (coagulative/liquefactive/caseous/enzymatic fat), atheromas/focal intimal injuries in aorta and larger arteries or ageing heart valves. Dystrophic calcification occurs despite normal calcium metabolism.
2. **Metastatic calcification:** Deposition of calcium in viable tissue, eg, blood vessels, kidneys, lungs and gastric mucosa. Metastatic calcification has the same morphology and pathogenesis as dystrophic calcification; however, it is always seen in a background of deranged calcium metabolism (hypercalcaemia). Causes of metastatic calcification include:
   - Hyperparathyroidism and hyperthyroidism
   - Vitamin-D intoxication
   - Systemic sarcoidosis (macrophages activate vitamin D precursor)
   - Milk–alkali syndrome (excessive calcium ingestion with antacids and milk)
   - 'Williams syndrome' or idiopathic hypercalcaemia of infancy (hypersensitivity to vitamin D)
   - Renal failure (causes retention of phosphate leading to secondary hyperparathyroidism)
   - Increased bone catabolism associated with disseminated bone tumours

Pathogenesis of Pathological Calcification

Pathological calcification has two major phases:

- **Initiation:** may occur in
  - Extracellular sites in membrane-bound vesicles 200 nm in size. Calcium is concentrated in these vesicles due to its affinity for acidic phospholipids.
  - Intracellular sites in mitochondria.
- **Propagation:** involves the formation of crystals of calcium hydroxyapatite.

Morphology of Pathological Calcification

Gross Pathology

Appears as fine white granules or clumps of gritty deposits.

Microscopy

- Seen on Hematoxylin and Eosin (H&E) sections as intracellular or extracellular basophilic amorphous granular deposits
- Sometimes single necrotic cells act as seeds which get encrusted with lamellar mineral deposits ('**psammoma body**') labelled so due to resemblance to grains of sand and commonly seen in some papillary cancers, eg, thyroid and meningiomas (Fig. 1.19)
- Calcium and iron salts may gather about long slender spicules of asbestos in lung, creating beaded, dumb-bell forms called '**asbestos bodies**'

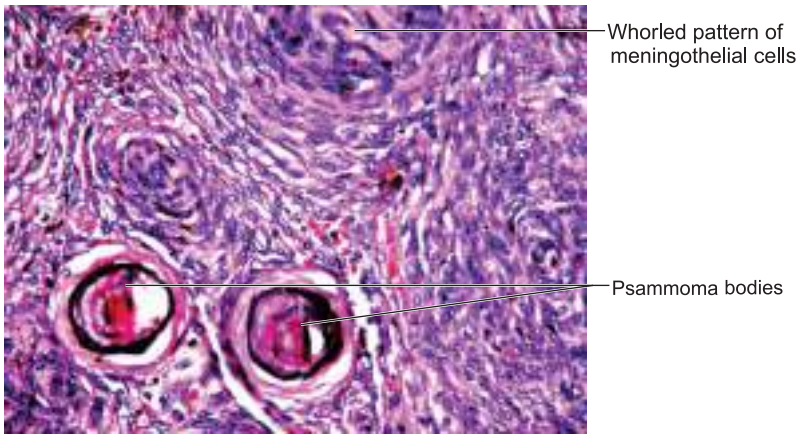


FIGURE 1.19. Dystrophic calcification (psammoma bodies) in a meningioma (200×).

Q. Differentiate between dystrophic and metastatic calcification.

Ans. Differences between dystrophic and metastatic calcification are shown in Table 1.4.

TABLE 1.8. Differences between dystrophic and metastatic calcification

Features	Dystrophic calcification	Metastatic calcification
Definition	Deposits of calcium salts in dead and degenerated tissue	Deposits of calcium salts in viable tissue
Calcium metabolism	Normal	Deranged
Serum calcium level	Normal	Increased
Sites of deposition	Necrosis, infarcts, thrombi, haematomas, dead parasites, old scars, atheromas	Blood vessels, kidneys, lungs and gastric mucosa

Q. Define cell ageing. Enumerate the biochemical and morphological alterations that occur during ageing.

Ans. Cell ageing is defined as loss of functional capacity and progressive decline in proliferative capacity, which ends in cell death.

Factors Contributing to Cell Ageing

- Genetic factors
- Diet
- Social conditions
- Atherosclerosis
- Diabetes mellitus
- Age-related diseases, eg, osteoarthritis

Indicators of Declining Cell Function Associated With Ageing

- Decreased oxidative phosphorylation
- Decreased synthesis of
  - Structural and enzymatic proteins
  - Cell receptors
- Decreased capacity for uptake of nutrients
- Decreased repair of chromosomal damage

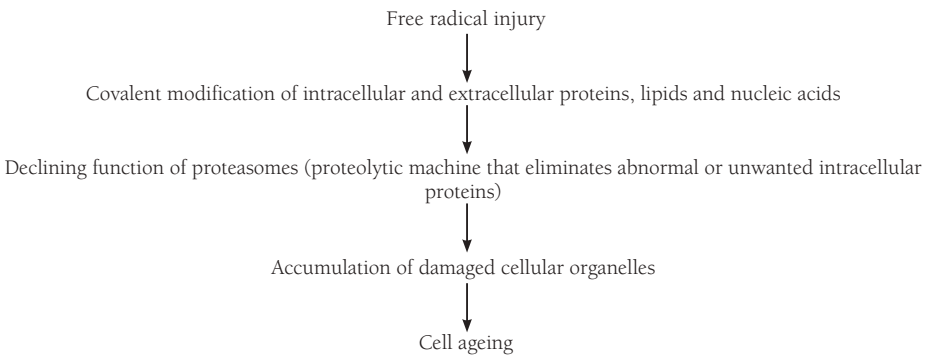
Morphologic Alterations due to Cell Ageing

- Irregular and abnormal location of nuclei
- Pleomorphic and vacuolated mitochondria
- Dilated and distorted endoplasmic reticulum
- Distorted Golgi apparatus

Theories of Cell Ageing

Cell ageing is considered to be multifactorial in origin. Factors influencing cell ageing include:

1. **Endogenous molecular programme of cellular senescence:**
   (a) Normally, DNA damage is repaired by DNA repair enzymes.
   (b) Accumulation of DNA damage due to defective DNA repair mechanisms induces ageing.
   (c) Also, contribution from activation of senescence-inducing apoptotic genes (on chromosomes 1 and 4) and induction of growth inhibitors.
   (d) Telomeres are critical for stabilization of terminal portion of chromosomes and anchoring them to the nuclear matrix. De novo synthesis of telomeres is regulated by an enzyme called *telomerase*. During somatic cell replication, a small segment of the telomere is not duplicated leading to *telomere shortening* and loss of DNA, inducing cellular ageing.
   (e) Telomerase repairs the shortened tips of chromosomes and maintains their length.
   (f) Repetitive mitoses (60–70 times) → telomeres lost → cell ageing.
   (g) Telomerase activity upregulated → telomere length maintained → avoids cell ageing.
2. **Exogenous influences (**Flowchart 1.15**):**



FLOWCHART 1.15. Exogenous influences in cellular ageing.

Q. What are heat shock proteins (HSPs)?

Ans. HSPs were so labelled because they were found in fruit fly larvae after slight elevation of temperature. They are essential to cell survival in species subjected to injury. There are two families of HSP—HSP 70 and HSP 60.

- HSP are involved in intracellular protein folding and translocation as well as targeting of proteins to their final destination. They are therefore also called chaperones or chaperonins.
- Their levels increase in stress.

Ubiquitin

- It is a small HSP critical to protein degradation (proteins degraded in cellular incinerators called 'proteasomes' when denatured beyond repair)
- Ubiquitin is universally or ubiquitously present in cells

2

Acute and Chronic Inflammation

Q. Define inflammation.

Ans. Inflammation is a complex reaction to injury that comprises 'vascular responses' and 'migration and activation of leukocytes'. It basically starts as the body's defence reaction, but may turn potentially harmful.

Q. What are the different stimuli for inflammation?

Ans. Stimuli for inflammation include

1. **Physical agents**: heat, radiation and mechanical trauma
2. **Chemical agents**: organic and inorganic poisons
3. **Infectious agents**: bacteria, viruses and parasites
4. **Immunological agents**: hypersensitivity reactions

Q. What are the cardinal signs of inflammation?

Ans. Cardinal signs of inflammation:

1. Rubor (redness)
2. Tumour (swelling)
3. Calor (heat)
4. Dolour (pain)

} Proposed by Celsus in the first century AD

5. Functio laesa (loss of function)–added later by Virchow

Q. What are the different types of inflammation?

Ans. Inflammation can be acute or chronic:

1. **Acute:** It is a transient process, which occurs within minutes of injury, lasts for hours or days and represents the early body reaction. It is usually followed by repair, a process by which tissue is restored to its original state as far as possible.
2. **Chronic:** It occurs when the causative agent of acute inflammation persists for a long time. Fibrosis and tissue necrosis usually accompany chronic inflammation.

Differences between acute and chronic inflammation are listed in Table 2.1.

Q. What are the major components of acute inflammation?

Ans. There are two major components of acute inflammation:

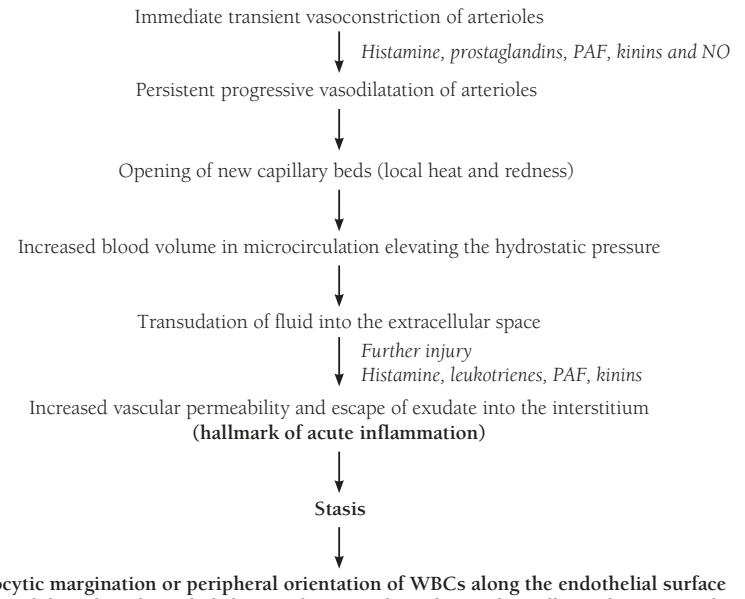
1. **Vascular events**
   (a) Alterations in vascular calibre that lead to an increase in blood flow
   (b) Structural changes in microvasculature, which permit plasma proteins and leukocytes to leave the circulation
2. **Cellular events**: Immigration of leukocytes from microcirculation and their accumulation in the focus of injury

TABLE 2.1. Comparison between acute and chronic inflammation

Feature	Acute	Chronic
Causative agents	Physical (heat, radiation and mechanical trauma) Chemical agents (organic and inorganic poisons) Infectious agents (bacteria, viruses and parasites) Immunological agents (hypersensitivity reactions)	Persistent acute inflammation due to nondegradable pathogens Persistent foreign bodies Autoimmune reactions
Major cells involved	Mainly neutrophils; also eosinophils and basophils	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells) and fibroblasts
Primary mediators	Vasoactive amines, eicosanoids	Interferon gamma (IFN-γ) and other cytokines, growth factors, reactive oxygen species, and hydrolytic enzymes
Onset	Immediate/rapid	Insidious/delayed
Duration	Few days	Up to many months or years
Outcomes	Resolution, fibrosis and chronic inflammation	Tissue destruction and scarring
Cardinal signs and systemic manifestations	1. Pain (dolour)	Absence of any cardinal signs
	2. Heat (calor)	Patient is asymptomatic or presents with low-grade fever, lethargy, loss of appetite and weight loss
	3. Redness (rubor)	Patient may also present with high-grade fever
	4. Swelling (tumour)	
	5. Loss of function (functio leasa)	
Oedema	Present	Absent
Angiogenesis	Absent	Present
Tissue destruction	Absent	Present
Attempts at repair	Absent	Present
Fibrosis	Absent	Present

Q. Write briefly on the vascular events in acute inflammation.

Ans. Vascular events in acute inflammation occur in the sequence shown in Flowchart 2.1.



FLOWCHART 2.1. Vascular events in acute inflammation.

Q. Enumerate the causes of leaky endothelium.

Ans. Causes of leaky endothelium include (Fig. 2.1A–E)

1. **Formation of endothelial gaps (immediate transient response)**
   (a) Affects venules 20–60 microns in diameter
   (b) Mediators (histamine, leukotrienes, etc.) cause cytoskeletal proteins to contract, leading to separation of intercellular junctions.
   (c) Cytokine mediators, like IL-1 and TNF, bring about cytoskeletal reorganization, causing endothelial retraction.
   (d) This response occurs rapidly after exposure to mediators.
   (e) It is reversible and short-lived (15–30 min).
2. **Direct endothelial injury (immediate sustained response)**
   (a) Toxins, burns, chemicals and bacterial infections result in endothelial cell necrosis, detachment and direct damage in the lumen (leading to thrombosis).
   (b) Leakage starts immediately and sustains for several hours.
   (c) All levels of circulation are affected.
3. **Delayed prolonged leakage**
   (a) Begins after a delay of 2–12 h, lasts for several hours or even days.
   (b) Involves venules and capillaries.

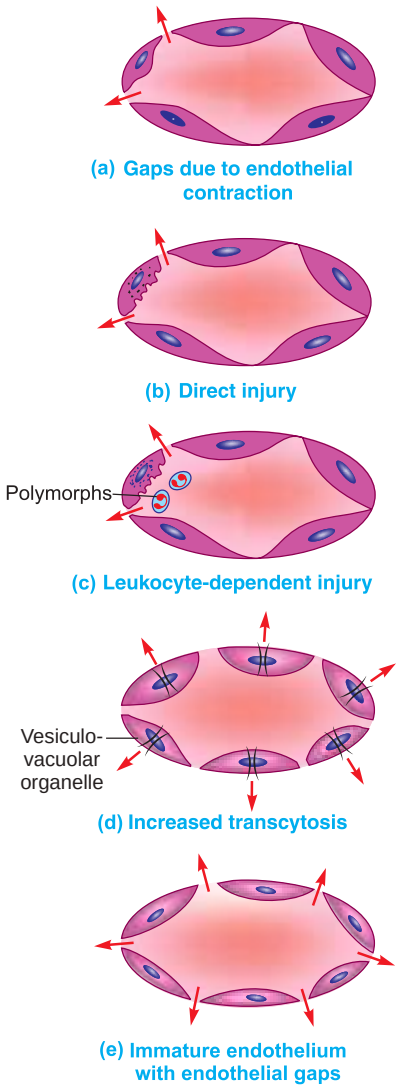


FIGURE 2.1. Causes of leaky endothelium. (A) Gaps due to endothelial contraction. (B) Direct injury. (C) Leukocyte dependant injury. (D) Increased transcytosis. (E) Immature endothelium with endothelial gaps.

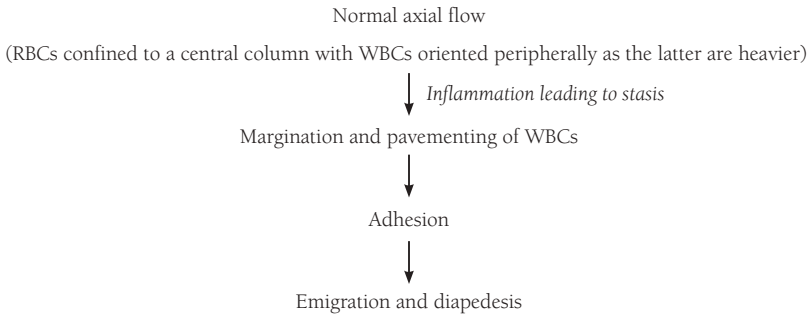
(c) Caused by thermal injury, X-rays, UV radiation and bacterial toxins, which lead to delayed endothelial damage by apoptosis or cause endothelial retraction by releasing cytokines.

4. **Leukocyte-mediated endothelial injury**
   (a) Leukocytes adhere to endothelium, release toxic oxygen species and proteolytic enzymes, which cause endothelial injury and increased permeability.
   (b) Largely restricted to venules, pulmonary and glomerular capillaries where leukocytes adhere for prolonged periods.
5. **Increased transcytosis across the endothelial cytoplasm**
   (a) Occurs across interconnected channels made of vesicles and vacuoles called vesiculovacuolar organelles.
   (b) Certain factors, eg, vascular endothelial growth factor (VEGF), appear to cause vascular leakage by increasing the number and size of the vascular channels.
6. **Leakage from new blood vessels**
   New vessels formed during angiogenesis remain leaky until endothelial cells mature and form intracellular junctions.

Q. Write briefly on the cellular events involved in acute inflammation.

Ans. The two main cellular events involved in acute inflammation are

1. **Extravasation of leukocytes** (movement from vessel lumen to interstitial space; Flowchart 2.2; Fig. 2.2).



FLOWCHART 2.2. Sequence of cellular events in acute inflammation.

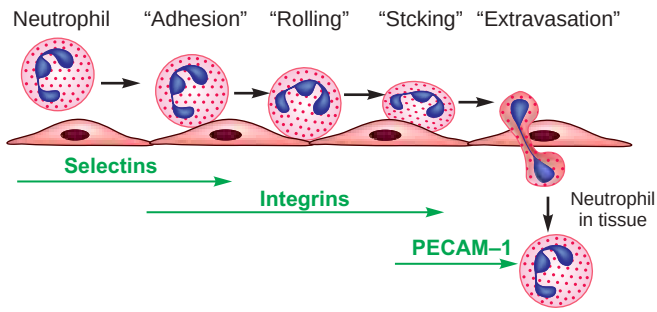


FIGURE 2.2. Mechanism of extravasation of leukocytes.

2. **Phagocytosis** (leukocytic engulfment of microbes, foreign particles, and cellular debris).
   Extravasation of leukocytes has the following steps:
   (a) **In the lumen: margination** (peripheral orientation of leukocytes), **rolling** (weak attachment of leukocytes to endothelium, detachment and binding again, causing a rolling movement), **pavementing or adhesion** (activation of leukocytes and firm binding of leukocytes to endothelium)

(b) **Transmigration across the endothelium** (**emigration or diapedesis**): emigration is facilitated by focal dissolution of the exposed basement membrane by leukocyte-derived collagenase
(c) **Migration** in interstitial tissue towards a chemotactic stimulus (**chemotaxis**)

Q. What are leukocyte adhesion molecules (LAMs)?

Ans. LAMs are molecules on the leukocytes and endothelial surfaces, which regulate leukocyte adhesion and transmigration.

- Chemical mediators affect the process of adhesion and transmigration by modulating the surface expression and avidity of adhesion molecules.
- LAMs are synthesized by endothelial cells and leukocytes.
- LAMs belong to four molecular families—**selections, immunoglobulin super family, integrins and mucin-like glycoproteins**.
- There are three types of selectins—L-selectins (expressed on leukocytes); E-selectins (expressed on endothelial cells) and P-selectins (expressed on platelets and endothelium). The ligands for selectins are sialylated oligosaccharides bound to mucin-like glycoproteins. TNF and IL1 increase endothelial expression of ligands for integrins like VCAM-1 (vascular cell adhesion molecule-1) which is the ligand for β1 integrin VLA-4 and ICAM-1 (intercellular adhesion molecule-1) which is the ligand for β2 integrins like LFA-1 and MAC-1.
- Corticosteroids inhibit adhesion molecule synthesis, thereby decreasing neutrophil adhesion and increasing the circulating absolute neutrophil count.
- Endotoxins enhance neutrophil adhesion, leading to a reduction in peripheral blood absolute neutrophil count.
- **Endothelial molecule and complimentary leukocyte molecule involved in rolling:**

Endothelial molecule (selectins)	Complimentary leukocyte molecule for selectins
P-selectin (CD 62P)	Sialyl-Lewis X-modified proteins
E-selectin (CD 62E)	Sialyl-Lewis X-modified proteins
GlyCam-1 (CD34)	L-selectin (CD62L)

- **Endothelial molecule and complimentary leukocyte molecule involved in adhesion:**

Endothelial molecule (integrins)	Complimentary leukocyte molecule for integrins
ICAM-1 (immunoglobulin family)	CD 11b/CD18 (β2) integrin (LFA-1, MAC-1)
VCAM-1 (immunoglobulin family)	VLA-4 (β1) integrin
GlyCam-1 (CD34)	L-selectin (CD62L)

- CD31 or platelet endothelial cell adhesion molecule-1 (PECAM-1) is the molecule involved in diapedesis.
- **There are three main types LAM deficiencies:**
  - **LAM deficiency type I** (integrin defects which present with recurrent bacterial infections, usually *Staphylococcus aureus* and gram-negative enteric bacteria. There is sustained leukocytosis due to the absence of leukocyte margination and the patient gives a history of delayed umbilical stump separation)
  - **LAM deficiency type II** (selectin defects which manifest with recurrent infections, Bombay phenotype and mental retardation)
  - **LAM deficiency type III** (mutations in the gene FERMT3 leading to impaired integrin activation resulting in recurrent infections, leukocytosis and petechial haemorrhage)

Q. Write briefly on chemotaxis.

Ans. Chemotaxis is defined as a locomotion oriented along a chemical gradient. All granulocytes, monocytes and to some extent lymphocytes exhibit directed movement to the area of injury, which is facilitated by chemotactic agents (chemoattractants).

Chemoattractants

1. **Exogenous**—bacterial products
2. **Endogenous**—C5a, leukotrienes and cytokines

Mechanism of chemotaxis (Flowchart 2.3; Fig. 2.3)

Binding of chemoattractants to **specific transmembrane G protein–coupled receptors (GPCRs) on the surface of leukocytes**

↓

Recruitment of G protein due to signals from GPCRs

↓

Activation of **phospholipase C (PLC), phosphoinositol 3 kinase (PI3K) and protein tyrosine kinases (effector molecules)**

↓

PLC + PI3K act on **membrane phospholipids**

↓

Generation of **lipid second messengers**

↓

↑ Cytosolic calcium

↓

Activation of **GTPases and numerous kinases**

↓

Polymerization of actin

↓

Locomotion

FLOWCHART 2.3. Mechanism of chemotaxis.

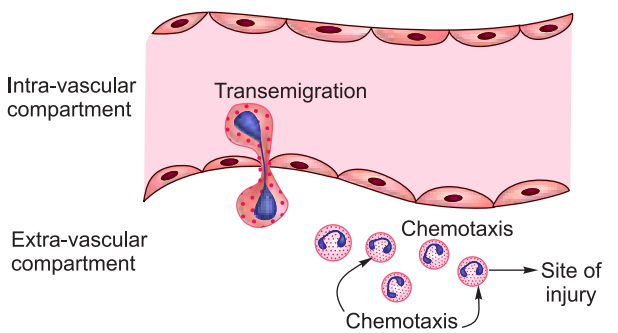


FIGURE 2.3. Mechanism of chemotaxis.

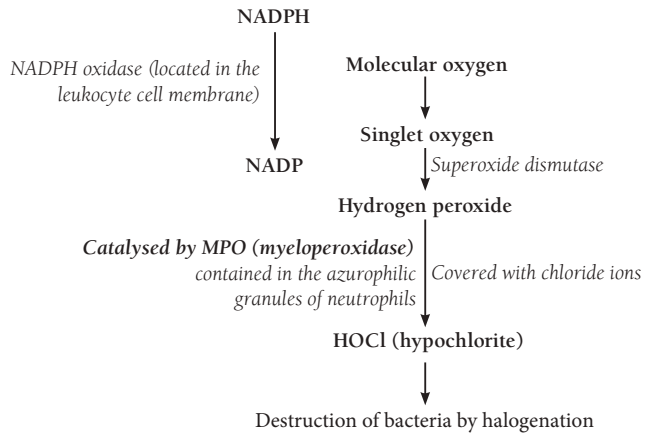
Q. Write briefly on phagocytosis.

Ans. Phagocytosis is defined as leukocytic engulfment of microorganisms, foreign particles and cellular debris. The two most important phagocytic cells are:

1. Polymorphs
2. Circulating monocytes or macrophages

Steps in Phagocytosis (Fig. 2.4)

1. **Recognition and attachment**
   (a) Typically, phagocytosis is initiated by recognition of the microorganisms and particles by receptors expressed on the leukocyte surface.
   (b) **Mannose receptors** and **scavenger receptors** are two important receptors that function to bind and ingest microbes. Mannose/fucose residues are typically a part of the microbial cell wall; whereas, mammalian cells instead contain sialic acid and N-acetylgalactosamine residues. Mannose receptors, therefore, recognize only the microbe and not the host cell. Macrophage scavenger receptors bind a lot of microbes.
   (c) The efficiency of phagocytosis is greatly enhanced by **opsonization** of bacteria (or foreign material).
   (d) The process of coating of a particle, such as a microbe, to target it for phagocytosis is called **opsonization** and the substances that do this are called **opsonins**. Phagocytes express high-affinity receptors for opsonins.
   (e) Major opsonins are '**IgG antibodies**', '**C3b breakdown products of complement**' and **plasma carbohydrate-binding lectins called** '**collectins**', which bind to the microbial cell wall sugar groups.
   (f) Leukocytes express **receptors for opsonins** that facilitate phagocytosis of the coated microbes, eg, **Fc receptor for IgG** (**FcγRI**), **complement receptors 1 and 3** (**CR1 and 3**) **for complement fragments** and **C1q for the collectins**.
2. **Engulfment**
   (a) Bacteria are engulfed by **pseudopodia** (**extensions of cytoplasm**) and trapped within phagosomes forming a phagocytic vacuole.
   (b) The limiting membrane of the phagocytic vacuole fuses with the limiting membrane of the lysosomal granule, resulting in discharge of the contents of the granule into the phagolysosome.
3. **Killing and degradation**
   (a) Neutrophils and monocytes are armed with both '**oxygen-dependent**' (**MPO system** and **O_2-derived free radicals**; Flowchart 2.4) as well as '**oxygen-independent**' (**lysosomal enzymes** and **reactive nitrogen species**, mainly derived from nitric oxide) mechanisms for killing bacteria.



FLOWCHART 2.4. Mechanism of killing by MPO–H_2O_2–halide system.

(b) The **oxygen-dependent MPO system** is the most potent bactericidal mechanism available to neutrophils and monocytes.

Other constituents of leukocyte granules which are also capable of killing microorganisms include

- ***Bactericidal/permeability-increasing*** (causes phospholipase activation and degradation of phospholipids)
- ***Lysozymes*** (causes degradation of bacterial coat oligosaccharides)
- ***Major basic protein*** (important eosinophilic granule constituent, which is toxic to parasites)
- ***Defensins*** (peptides that kill microbes by creating holes in their membranes)
- ***Neutrophil extracellular taps or NETs*** (Extracellular fibrillary networks consisting a viscous meshwork of nuclear chromatin of neutrophils that trap the microbe at the site of infection by fibrils and prevent their spread)

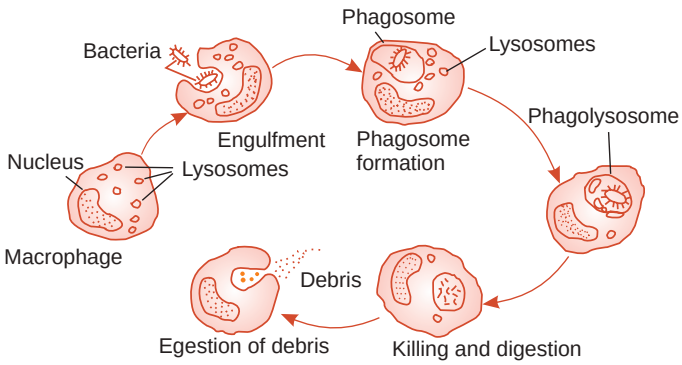


FIGURE 2.4. Mechanism of phagocytosis.

Q. Enumerate the defects in leukocyte functions.

Ans. Defects in leukocyte functions may be:

1. **Genetic**
   (a) **Chediak–Higashi syndrome:** disorder of lysosomal granules; prevents fusion of lysosomes with phagosomes to form phagolysosomes
   (b) **Chronic granulomatous disease of childhood:** X-linked/autosomal recessive disease characterized by absence of NADPH oxidase
   (c) **Myeloperoxidase deficiency:** absent MPO–H_2O_2 system
2. **Acquired**
   (a) **Defective chemotaxis**: thermal injury, diabetes, malignancy, sepsis and immunodeficiencies
   (b) **Defective adhesion:** haemodialysis and diabetes
   (c) **Defective phagocytosis and microbicidal activity**: leukaemia, anaemia, sepsis, diabetes, neonates and malnutrition

Q. Enumerate the chemical mediators of inflammation.

Ans. Chemical mediators of inflammation may be

1. **Cell derived**

Sources	Mediator	Action
Mast cells, basophils and platelets	Histamine[a]	Vasodilatation, increased permeability, endothelial activation, itching and pain
Platelets and enterochromaffin cells	Serotonin[a]	Actions like histamine but less potent
Neutrophils and macrophages	Lysosomal enzymes[a]	Tissue damage
All leukocytes and endothelial cells	Platelet-activating factor[b]	Increased vascular permeability
All leukocytes	Leukotrienes[b] (slow reacting substances of anaphylaxis)	**LTC4, LTD4 and LTE4** • Increased permeability of vessels • Smooth muscle contraction • Vasoconstriction • Bronchoconstriction **LTB4** • Chemotaxis • Cell adherence
All leukocytes, platelets and endothelial cells	Prostaglandins[b]	**PGD2 and PGE2** • Vasodilatation • Bronchodilatation • Increased permeability of vessels **PGF2α** • Vasodilatation • Bronchoconstriction **TXA2** • Vasoconstriction • Bronchoconstriction • Platelet aggregation **PGI2** • Vasodilatation • Bronchodilatation • Inhibition of platelet aggregation
Lymphocytes, macrophages and endothelial cells	Cytokines[b]	Increased leukocyte adherence, thrombosis, fibroblastic proliferation and acute phase reaction (**IL8** chemotactic for neutrophils, **PF4** chemotactic for neutrophils, monocytes and eosinophils, **MCP-1** chemotactic for monocytes and **eotaxin** chemotactic for eosinophils)
Macrophages	Nitric oxide[b]	Vasodilatation, antiplatelet effect and microbicidal action
Neutrophils and macrophages	Oxygen-derived free radicals	Endothelial damage and increased vascular permeability

[a]Preformed mediators.
[b]Newly synthesized mediators.

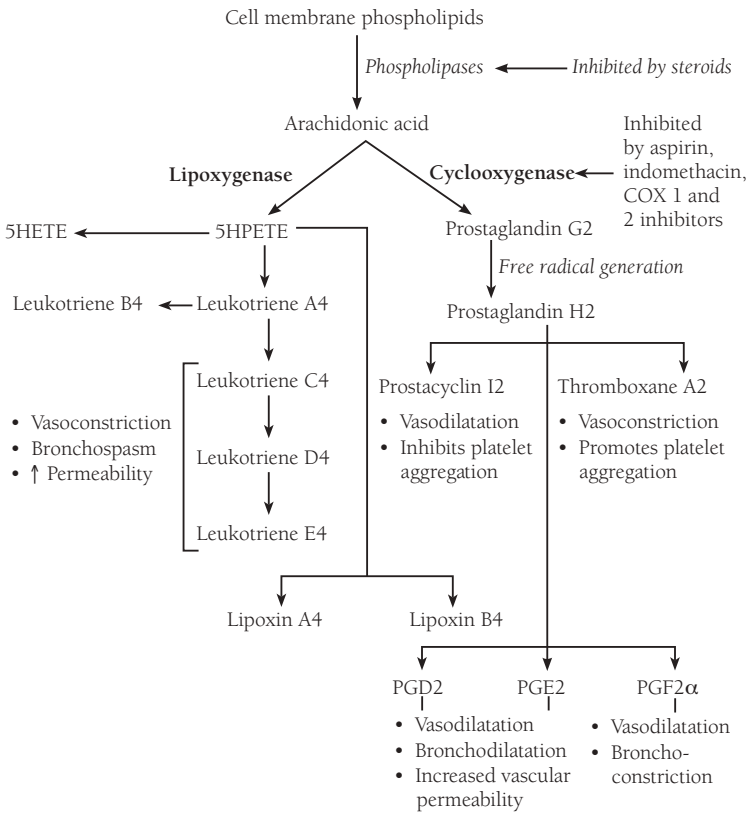
2. **Plasma derived**

Sources	Mediator	Action
Clotting and fibrinolytic system	Fibrin split products	Increased vascular permeability
Kinin system	Kinin/bradykinin	Increased vascular permeability
Complement system	Anaphylatoxins, C3a, C4a and C5a	Increased vascular permeability

- **Plasma-derived** mediators are mainly produced in the liver; circulate in precursor form and must be activated.
- **Cell-derived** mediators are synthesized de novo or are preformed and stored in intracellular granules and need to be secreted.
- **Production of active mediators** is triggered by microbial products or by host proteins, such as proteins of the complement, kinin and coagulation systems that are themselves activated by microbes and damaged tissue.
- Most mediators act by binding to specific receptors on target cells; one mediator can stimulate release of other mediators.
- Once activated and released from the cells, most of these mediators are short-lived.
- The **various mechanisms underlying their action** include
  - Receptor–ligand interactions
  - Direct enzymatic activity
  - Oxidative damage

Q. Enumerate the steps involved in generation of arachidonic acid metabolites.

Ans. Steps involved in generation of arachidonic acid metabolites and their role in inflammation are summarized in Flowchart 2.5.



HETE – Hydroxyeicosatetraenoic acid
HPETE – Hydroperoxyeicosatetraenoic acid

FLOWCHART 2.5. Generation of arachidonic acid metabolites and their role in inflammation.

- There are two types of cyclooxygenases–COX-1 and COX-2. COX-1 are responsible for the production of prostaglandins which are involved in both inflammation and homoeostasis (fluid and electrolyte balance and cytoprotection of gastrointestinal tract or GIT), whereas COX-2 generate prostaglandins that are only involved in inflammation. The role of COX-2 inhibitors has therefore been explored as anti-inflammatory agents. It has

been found that COX-2 may not be completely selective and may also play a role in homoeostasis and also COX-2 inhibitors may increase the risk of cardiovascular and cerebrovascular events as they decrease endothelial production of Prostacyclin-I-2 (vasodilator and inhibitor of platelet aggregation).
- Lipoxygenase is not affected by non-steroidal antiinflammatory drugs (NSAIDs). Inhibitors of this enzyme may be helpful in asthma as they inhibit production of leukotrienes.

Q. Write briefly on platelet-derived factor (PAF).

Ans. PAF is a bioactive phospholipid-derived mediator which has multiple inflammatory effects. It mediates its effects via a single G protein–coupled receptor and is **regulated** by a family of inactivating PAF acetyl hydrolases.

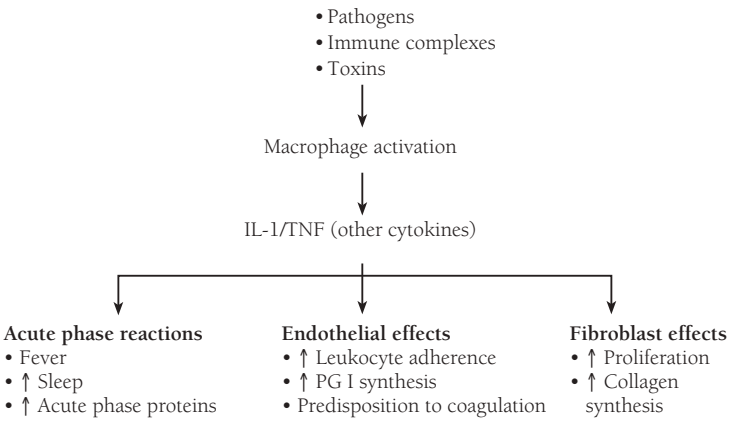
- **Sources:**
  - Endothelial cells
  - Platelets
  - Neutrophils, basophils and monocytes
- **Actions/effects:**
  - Vasoconstriction
  - Bronchospasm
  - Leukocyte adhesion to endothelium
  - Chemotaxis
  - Degranulation
  - Oxidative burst
  - Synthesis of mediators, eg, eicosanoids

Q. What are cytokines? Describe their role in inflammation.

Ans. Cytokines are messenger proteins secreted by some cell types (activated lymphocytes, macrophages, endothelial, epithelial and connective tissue cells) which modulate the function of other cell types.

- **TNF and IL-1 are the prototypes.**
- TNF mediates the effects of septic shock (causes hypotension, ↓ vascular resistance, ↑ heart rate and ↓ blood pH). It was earlier classified into TNF-α and -β. Presently, TNF-α is actually considered TNF; TNF-β is thought to be a lymphotoxin (produced by activated T lymphocytes). Secretion of TNF and IL1 is induced by:
  - Bacterial products
  - Immune complexes
  - Toxins
  - Cytokines
  - Physical injury

Major effects of IL-1 and TNF in inflammation are depicted in Flowchart 2.6.



FLOWCHART 2.6. Major effects of IL-1 and TNF in inflammation.

Q. Write briefly on chemokines.

Ans. Chemokines are a family of small, 8–10 kD proteins that act primarily as chemoattractants for specific leukocytes.

- They bind to seven transmembrane G protein–coupled receptors.
- There are 40 different types of chemokines and 20 different types of receptors.
- Chemokines are classified into four groups based on the arrangement of conserved cysteine residues in the proteins, namely,
  1. **CXC chemokines or α-chemokines**
     (a) One amino acid residue separates first two conserved cysteine residues.
     (b) Typical example is IL8. IL8 is secreted by macrophages and endothelial cells and primarily acts on neutrophils (activation and chemotaxis of neutrophils).
  2. **CC chemokines or β-chemokines**
     (a) Two conserved cysteine residues are located adjacent to each other.
     (b) Include MCP-1, eotaxin, MIP-α and RANTES (regulated and normal T cell expressed and secreted).
     (c) Chemotactic for monocytes, eosinophils, basophils and lymphocytes (not neutrophils).
  3. **C chemokines or γ-chemokines:** Lack first and third (two of four) cysteines, eg, lymphotactin specific for lymphocytes.
  4. **CX_3C chemokines:** Three amino acid residues between two cysteines, eg, fractalkine.

Q. Write briefly on nitrous oxide (NO).

Ans. NO (also called endothelium-derived relaxing factor) is a soluble gas produced by endothelial cells (eNOS), macrophages (iNOS) and neurons (nNOS).

- Of these isoforms, eNOS is constitutively expressed (activated rapidly by increase in calcium).
- iNOS is induced when macrophages are activated by cytokines (TNF, γIFN).
- NO is synthesized from L-arginine by NOS (nitric oxide synthase).
- Acts in a paracrine manner via cyclic GMP.
- Causes vasodilatation, ↓ platelet aggregation, ↓ platelet adhesion, ↓ mast cell-induced inflammation.
- Regulates leukocyte recruitment and microbicidal activity.

Q. Write briefly on neuropeptides.

Ans. Neuropeptides are small proteins such as substance P and neurokinin A that transmit pain signals, regulate vessel tone and modulate vascular permeability. They are secreted by nerve fibres in lungs, GIT and leukocytes.

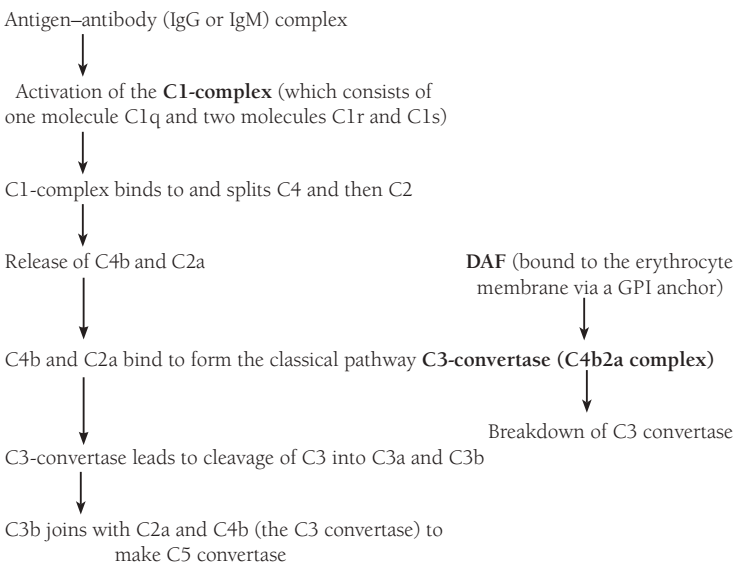
Q. Write briefly on complement system.

Ans. Complement was first discovered as a **heat-labile component of normal plasma** that was found to **add to** or **augment the opsonization of bacteria by antibodies** and **facilitate bacterial killing**. The name derives from the fact that this system was found to 'complement' the antibacterial activity of the antibody.

- Complement system is an **important part of the innate** and **adaptive immune responses**.
- It is constituted by a **collection of 30 different proteins** which include serum and cell surface proteins as well as cell membrane receptors.
- The complement proteins, in their precursor zymogen forms, are **widely distributed throughout body fluids and tissues without any adverse effect**. They are activated at

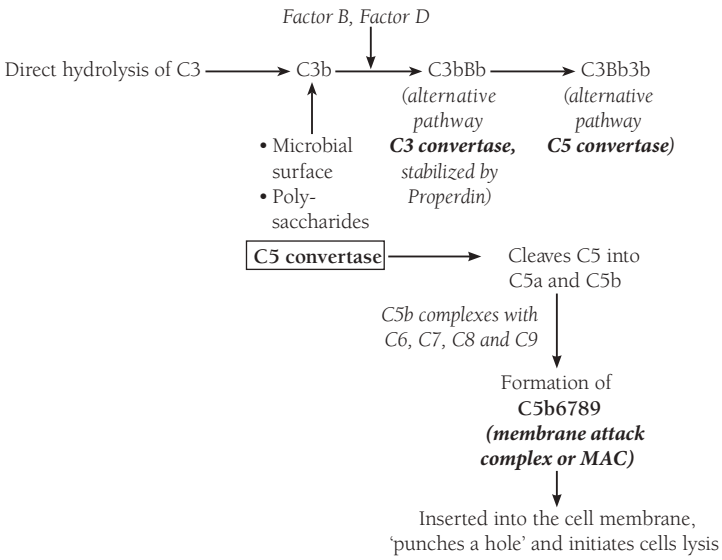
sites of infection or injury, however, to trigger a series of potent inflammatory events. Their action occurs via **three pathways**:

1. **Classical pathway** (Flowchart 2.7)



FLOWCHART 2.7. Classical pathway of complement activation.

2. **Alternative pathway** (Flowchart 2.8)



FLOWCHART 2.8. Alternative pathway of complement activation.

3. **Mannose-binding lectin pathway or MBL–MASP** (MBL-associated serine proteases; homologous to the classical pathway; Flowchart 2.9):

Binding of MBL to mannose residues on pathogen surface
↓
Activation of the MBL-associated serine proteases, MASP-1 and MASP-2 (very similar to C1r and C1s, respectively)
↓
Splitting of C4 into C4a and C4b and C2 into C2a and C2b
↓
Binding together of C4b and C2a to form the C3-convertase as in the classical pathway

FLOWCHART 2.9. Complement activation by MBL–MASP pathway.

Effector Functions of Complement Proteins

- **C3b:** Opsonization
- **C5a:** Chemotaxis
- **C3a** and **C5a:** Increased permeability of the capillary beds
- The early complement components break down and eliminate the antigen-antibody complexes from the body, failure of which can lead to immune complex diseases.
- **Killing of microbes through direct lysis is mediated by the MAC, C5b-9** (Complement-mediated lysis can cause serious disorders such as Rh disease, immune haemolytic anaemia and immune thrombocytopenic purpura).
- **Complement promotes antibody formation through breakdown products.** Breakdown of C3b generates a fragment (C3d) that binds to antigens to aid in their uptake by antigen-presenting cells and B cells.

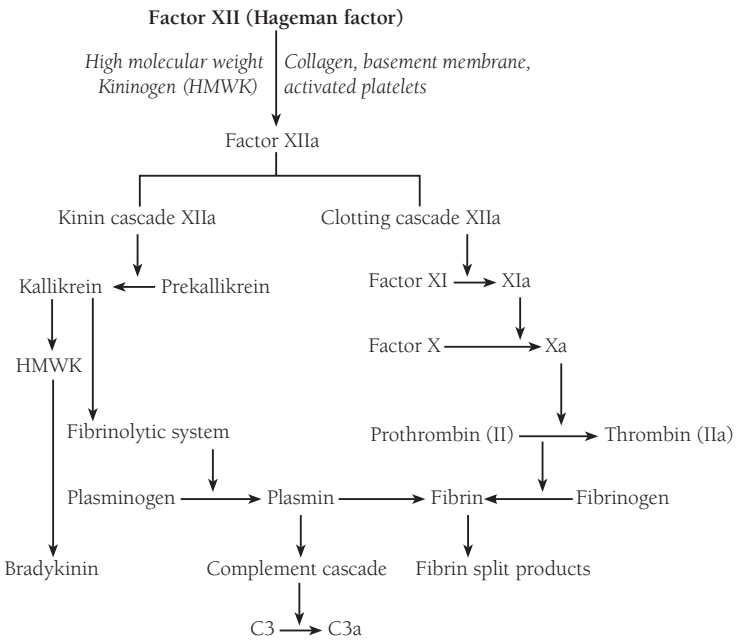
Q. Enumerate the steps involved in activation of kinin and clotting systems.

Ans. Activated Hageman factor initiates four interrelated systems (Flowchart 2.10), **namely,**

- Kinin system
- Clotting system
- Fibrinolytic system
- Complement system

Activation of **prekallikrein activator** by factor XIIa eventually generates **bradykinin**, which **has the following functions:**

- Smooth muscle contraction
- Vasodilatation
- Increased vascular permeability
- Generation of pain



FLOWCHART 2.10. Interrelationship of four plasma-derived systems (Hageman factor XII of the clotting system plays a key role in the interaction of these systems).

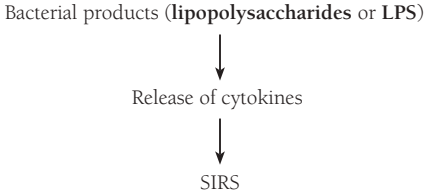
Q. Enumerate the factors determining variation in inflammatory response of different individuals.

Ans. Factors determining variation in individual inflammatory responses are as follows:

1. **Factors involving the organism:**
   (a) Type of injury
   (b) Virulence and dosage of infective organism
   (c) Portal of entry
2. **Factors involving the host:**
   (a) General health of host; starvation, chronic debilitating diseases like diabetes mellitus and alcoholism render the host more susceptible to infections
   (b) Immune status of host
   (c) Neutropenia
   (d) Site or type of tissue involved
   (e) Local host factors—ischaemia, presence of foreign bodies, necrosis, etc.

Q. What are the systemic effects of inflammation?

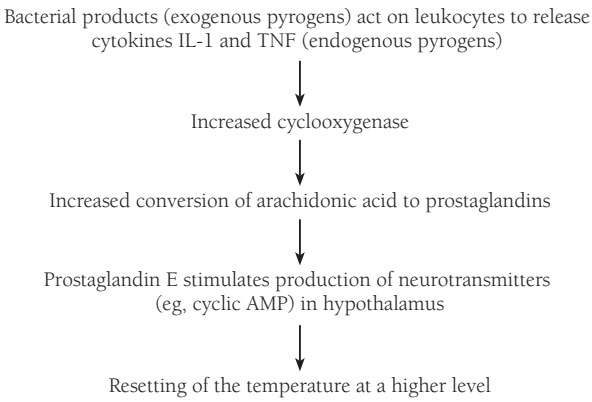
Ans. Systemic changes associated with inflammation are collectively called acute phase response or **systemic inflammatory response syndrome** (**SIRS**; Flowchart 2.11).



FLOWCHART 2.11. Systemic inflammatory response syndrome.

Manifestations of SIRS include

1. **Fever:** The mechanism underlying development of fever is depicted in Flowchart 2.12.



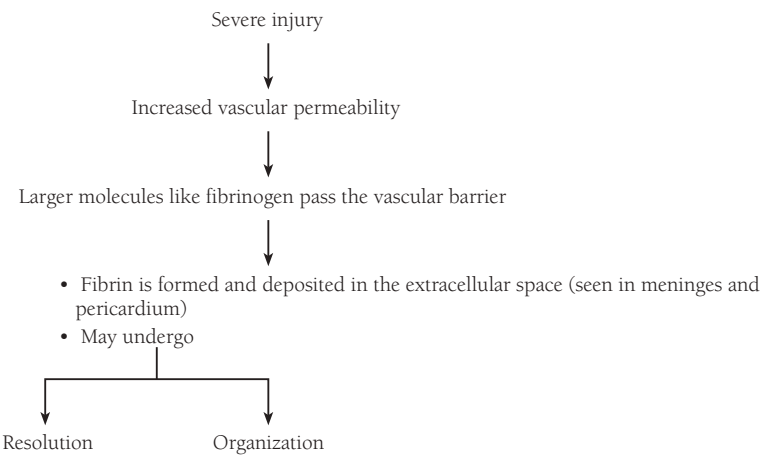
FLOWCHART 2.12. Mechanism of development of fever in SIRS.

2. **Release of acute phase proteins:** Acute phase proteins/reactants, eg, C reactive protein (CRP), fibrinogen and serum amyloid A (SAA) protein, are mostly synthesized in the liver. They bind to the cell wall (act as opsonins) and complement, and their synthesis is regulated by cytokines IL-1, IL-6 and TNF.
3. **Leukocytosis:** Accelerated release of cells from the bone marrow post-mitotic reserve pool with a shift to left (due to IL-1 and TNF) is common in inflammation and this may result in counts as high as 40,000–100,000 cells/μL (**leukaemoid reaction**).
4. **Other manifestations:** Increased pulse and blood pressure, decreased sweating, rigours, chills, anorexia, somnolence and malaise

Q. Enumerate and describe the various morphological patterns of acute inflammation?

Ans. Morphologic patterns of acute inflammation:

1. **Serous inflammation:** It is characterized by collection of a watery, protein-poor fluid; derived from either the plasma or secretions of mesothelial cells (lining peritoneal, pleural and pericardial cavities). It is usually associated with burns, viral infections, etc.
2. **Fibrinous inflammation** (Flowchart 2.13): Examples of fibrinous inflammation include 'bread and butter' pericarditis seen in acute rheumatic fever and fibrinous pleuritis.



FLOWCHART 2.13. Pathogenesis and outcomes of fibrinous inflammation.

3. **Suppurative inflammation:**
   - It is characterized by production of large amount of pus or purulent exudates comprising neutrophils, necrotic cells and oedema fluid. The pus may collect locally to form an **abscess** (abscesses, typically have a large central necrotic cavity rimmed by a layer of preserved neutrophils and may be surrounded by a zone of dilated vessels and proliferating fibroblasts).
   - Occurs secondary to infections with pyogenic (pus-producing organisms), eg, staphylococci.
4. **Catarrhal inflammation:** Also called phlegmonous inflammation, it is characterized by acute inflammation of the mucous membranes resulting in excessive mucous production (eg, running nose).
5. **Membranous inflammation:** This type of inflammation involves formation of a membrane over the epithelial surfaces. The membrane is constituted by fibrin, desquamated epithelial and inflammatory cells, eg, membrane formation is pharyngitis associated with *Corynebacterium diphtheriae*.

Q. Define cellulitis.

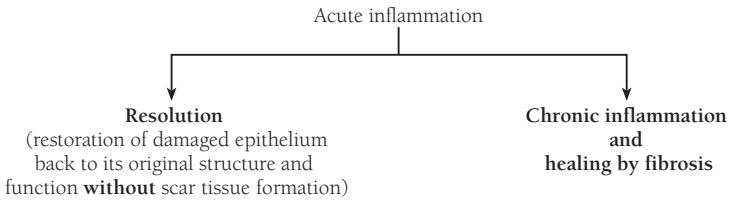
Ans. Cellulitis is caused by thin, watery exudate that spreads throughout subcutaneous tissue.

Q. Define an ulcer.

Ans. An ulcer is a local defect, or excavation on the surface of an organ or tissue that results due to sloughing of inflammatory necrotic material. During the acute stage, there is intense polymorphonuclear infiltration and vascular dilatation. With chronicity, the base and margins of the ulcer develop fibroblastic proliferation, scarring and infiltration by chronic inflammatory cells.

Q. What are the possible outcomes of acute inflammation?

Ans. Possible outcomes of acute inflammation are given in Flowchart 2.14.



FLOWCHART 2.14. Outcomes of acute inflammation.

Q. Define chronic inflammation.

Ans. Inflammation of prolonged duration (lasting weeks or months) is labelled chronic inflammation. It is characterized by three simultaneously ongoing components:

1. Active inflammation
2. Tissue destruction
3. Attempts at repair

Typically, chronic inflammation is low grade and associated with an asymptomatic clinical response.

Q. Enumerate the causes of chronic inflammation and write briefly on its morphology.

Ans.

Causes

1. **Persistent infections,** such as tuberculosis, syphilis, infections due to certain viruses, fungi and parasites. Typically, these organisms
   (a) Are of low toxicity.
   (b) Evoke an immune response called delayed hypersensitivity.
   (c) Are characterized by a specific inflammatory response called a **granulomatous** reaction.
2. **Prolonged exposure to potentially toxic agents:**
   Exogenous—Silica → Silicosis
   Endogenous—Toxic lipid components → Atherosclerosis
3. **Autoimmunity:** Immune reaction against one's own antigens can result in chronic tissue damage.

Morphologic Features of Chronic Inflammation

1. Infiltration by *mononuclear cells* (lymphocytes, macrophages and plasma cells)
2. *Tissue destruction* due to persistent offending agent/inflammation
3. *Healing* by connective tissue replacement of damaged tissue includes proliferation of blood vessels (angiogenesis and fibrosis)

Cells Involved in Chronic Inflammation

1. **Lymphocytes** are mobilized in antibody-mediated, cell-mediated as well as nonimmune inflammation (both T and B lymphocytes are involved; Fig. 2.5).

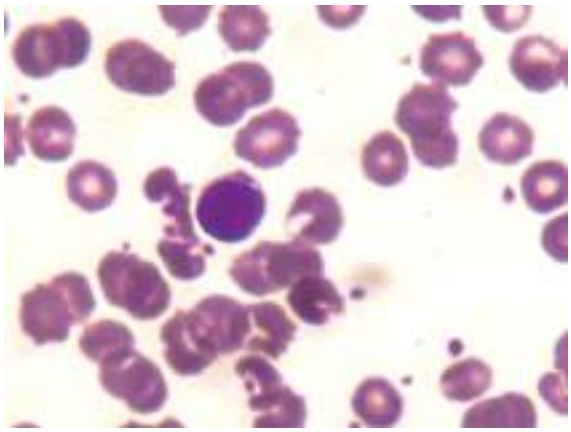
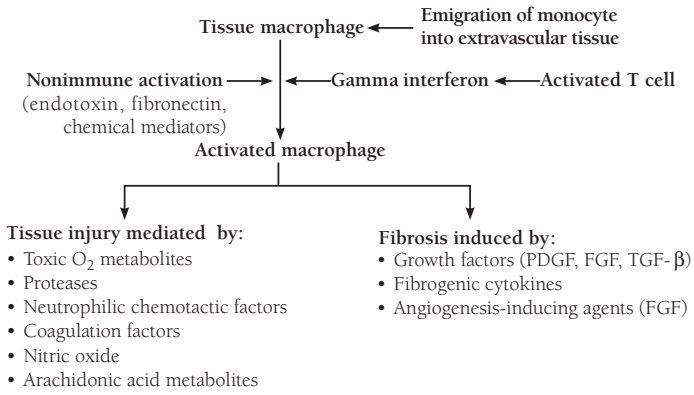


FIGURE 2.5. A small lymphocyte showing scanty basophilic, agranular cytoplasm; high N:C ratio and clumped nuclear chromatin.

2. **Macrophages** bring about phagocytosis, initiate tissue repair, secrete mediators of inflammation and influence lymphocyte function (interact with lymphocytes in chronic inflammation as shown in Flowchart 2.15).



FLOWCHART 2.15. Interactions of macrophages with lymphocytes in chronic inflammation.

3. **Eosinophil** (Fig. 2.6)
   - Recruitment and extravasation from blood driven by adhesion molecules like neutrophils and by **eotaxin** (chemokine derived from leukocytes and epithelial cells which is specific for eosinophils)
   - Involved in IgE-mediated immune reactions and parasitic infections
   - **Major basic protein** is a highly toxic protein contained in eosinophil granules. It is toxic to parasites and mammalian epithelial cells.

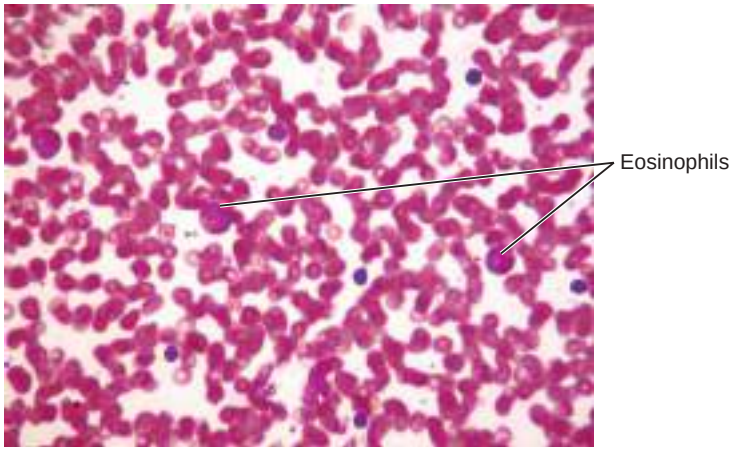


FIGURE 2.6. Eosinophil showing a bilobed nucleus and coarse red granules.

4. **Mast cells** (Fig. 2.7)
   - Widely present in connective tissue.
   - Participate in both acute and chronic inflammatory cells.
   - Express on their surface the receptor that binds to the Fc portion of IgE antibody to degranulate mast cells and release mediators (histamines and prostaglandins).

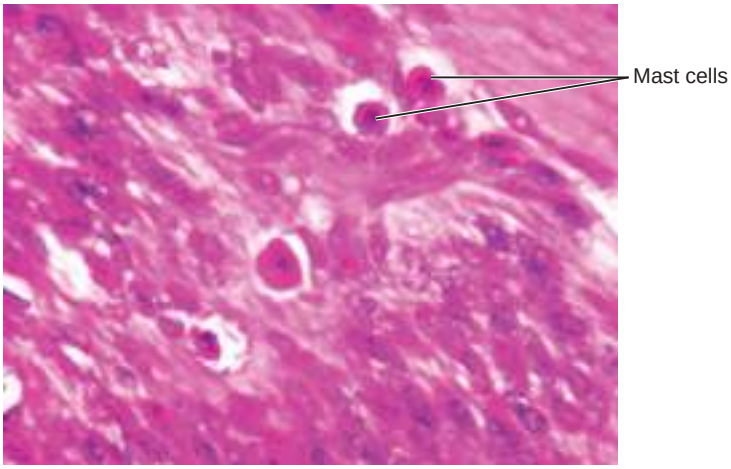


FIGURE 2.7. Mast cells showing abundant basophilic granules and round nuclei.

5. **Plasma cells** (Fig. 2.8)
   - Plasma cells are large cells with amphophilic to basophilic cytoplasm, an eccentric nucleus with the chromatin arranged in a characteristic cart-wheel or clock-face pattern.
   - Their cytoplasm contains a pale perinuclear zone that on electron microscopy shows an extensive Golgi apparatus and centrioles.
   - Plasma cells have abundant rough endoplasmic reticulum coupled with a well-developed Golgi apparatus, which together are responsible for immunoglobulin secretion.

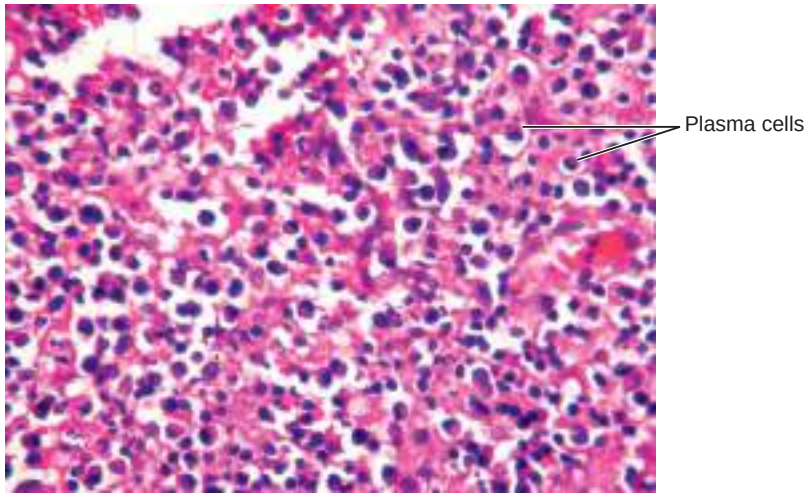


FIGURE 2.8. Plasma cells with amphophilic to basophilic cytoplasm, and an eccentric nucleus with heterochromatin arranged in a characteristic cart-wheel or clock-face arrangement and pale perinuclear zone.

6. **Neutrophils**

Although neutrophils have been classically associated with acute inflammation, they may sometimes be seen in chronic inflammation as well (recruited by mediators produced by activated macrophages and lymphocytes), eg, chronic osteomyelitis (bacterial infection of bone). This is labelled 'acute on chronic inflammation'.

Q. Enumerate the steps in mononuclear cell differentiation.

Ans.

Mononuclear Cell Differentiation

Stem cell ⟶ monoblast ⟶ monocyte/macrophage

Macrophages have different names in different tissues, eg, **'sinus histiocytes' in lymph node, 'osteoclasts' in bone, 'microglia' in central nervous system, 'Kupffer cells' in liver** and **'alveolar macrophages' in lung.**

Q. What is an activated macrophage?

Ans. An activated macrophage has the following salient features:

- Increased cell size
- Increased level of lysosomal enzymes
- Active metabolism
- Greater ability to phagocytose and kill
- Secretion of a large variety of biologically active products

Q. What is granulomatous inflammation? Enumerate some granulomatous diseases.

Ans. Granulomatous inflammation is a distinctive pattern of chronic inflammatory reaction characterized by the presence of granulomas. A granuloma is a microscopic aggregation of activated macrophages which transform into epithelioid (epithelial like) cells. Epithelioid cells have scanty to moderate, ill-defined, pale pink cytoplasm and a slipper-shaped vesicular nucleus. These are surrounded by a collar of mononuclear cells (lymphocytes and plasma cells). Older granulomas have an enclosing **rim of fibroblasts** and **connective tissue**. Epithelioid cells fuse to form '**giant cells**' 40–50 microns in size with 20 or more nuclei arranged peripherally like a horse shoe (**Langhans giant cells**; Fig. 2.9).

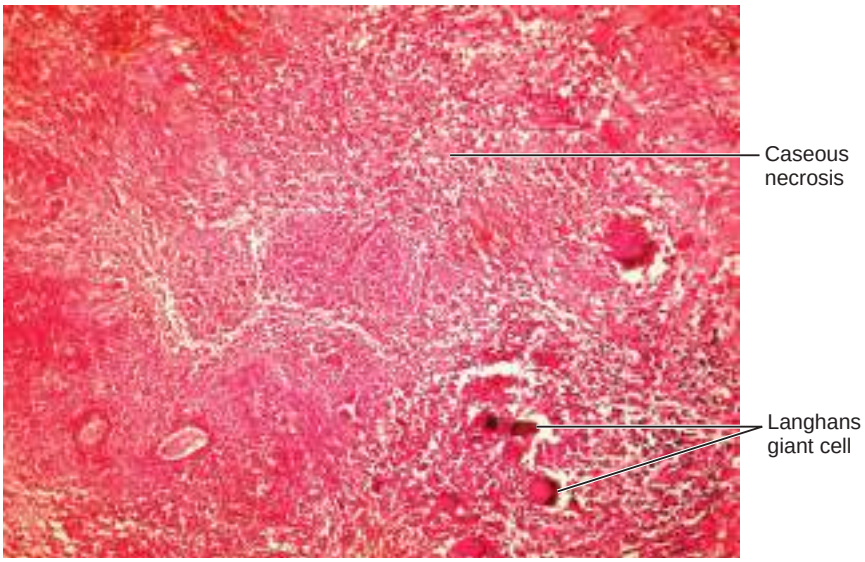


FIGURE 2.9. A caseating epithelioid cell granuloma with Langhans giant cells (H&E; 100x).

Types of Granulomas

1. **Infectious granulomas**
   (a) **Tuberculosis** (prototype of granulomatous disease): Caused by *Mycobacterium tuberculosis,* tuberculosis is associated with the formation of caseating granulomas (granulomas showing presence of central granular debris with loss of all cellular detail and higher positivity for acid fast bacilli) or noncaseating granulomas (absence of caseation and low positivity for acid fast bacilli).
   (b) **Leprosy:** It is caused by *Mycobacterium leprae*. Noncaseating granulomas are typically seen with or without acid fast lepra bacilli in the macrophages.
   (c) **Syphilis:** It is caused by *Treponema pallidum*. **Gumma** formation is the disease hallmark. Gumma is histopathologically characterized by a central necrotic area without loss of cellular outline; plasma cell infiltrate with a wall of histiocytes.
   (d) **Cat scratch disease:** It is caused by a Gram-negative bacillus. It typically shows rounded or stellate granulomas containing central granular debris and large number of neutrophils.
   (e) **Deep fungal infections:** Fungal granulomas are caused by organisms like *histoplasma* and *blastomyces* and are typically suppurative (granulomas with neutrophilic inflammation).
2. **Noninfectious or immune granulomas:** Granulomas form in response to persistent presence of nondegradable or particulate material, which incites an immune response. These are usually noncaseating epithelioid cell granulomas. Examples includes arcoidosis and hypersensitivity pneumonitis.
3. **Foreign body granulomas** are formed as a response to foreign bodies like talc, suture and intravenous drugs. The foreign material can be identified in the centre of the granuloma or within the foreign body giant cells which have a haphazard distribution of nuclei unlike Langhans giant cell.

Q. Enumerate the components of granulation tissue.

Ans. Granulation tissue (Fig. 2.10) has the following components:

1. Newly formed blood vessels (endothelial proliferation or neoangiogenesis)
2. Chronic inflammatory cells
3. Proliferating fibroblasts
4. Extracellular matrix which in comparison to ordinary extracellular matrix is more cellular and more vascular

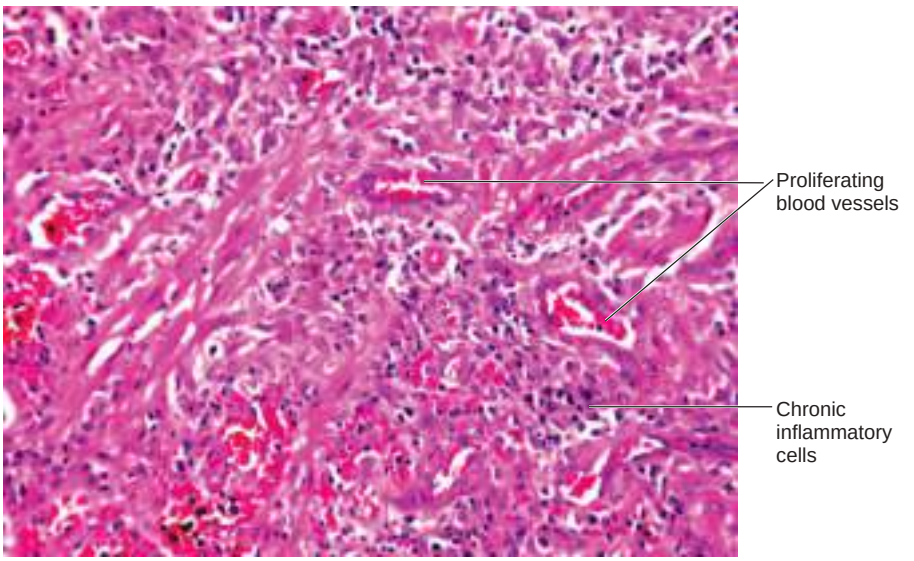


FIGURE 2.10. Section showing granulation tissue components (congested capillaries in an oedematous background with plasma cells, lymphocytes, histiocytes and fibroblasts).

Q. Differentiate between granulation tissue and granuloma.

Ans. Differences between granulation tissue and granuloma are shown in Table 2.2.

TABLE 2.2. Differences between granulation tissue and granuloma

Features	Granulation tissue	Granuloma
Component of	Healing and repair	Chronic inflammation; occurs due to delayed hypersensitivity response
Definition	Tissue composed of newly formed blood vessels (**angiogenesis**), proliferating fibroblasts and chronic inflammatory cells	Microscopic aggregation of macrophages that are transformed into epithelium like (**epithelioid cells**) surrounded by a collar of mononuclear cells (**lymphocytes and plasma cells**) Older granulomas have an enclosing **rim of fibroblasts and connective tissue**
Giant cells	Not seen	Epithelioid cells fuse to form '**Langhans giant cells**' 40–50 microns in size with 20 or more nuclei arranged peripherally
Remodelling (maturation and reorganization of fibrous tissue)	Seen	Not seen
Growth factors	Angiogenic and fibrogenic growth factors involved, eg, PDGF, FGF, TNF and VEGF	Involvement of cytokines like IL-1, IL-12 and γIFN

3

Healing and Repair

Q. Define repair.

Ans. Restoration of tissue architecture and function after injury is termed repair.

It may occur in two ways:

1. **Regeneration:** The injured tissue reverts to normal after replacement of damaged components by the active proliferation of residual cells as well as maturation of stem cells.
2. **Healing with scar formation:** If the individual tissue is incapable of complete restoration to original state or if there is severe damage to the supporting structures, repair occurs by a laying down of connective tissue. This is labelled 'healing with scar formation'.

Q. Define fibrosis.

Ans. A term used to describe extensive deposition of collagen that occurs in parenchymal organs as a consequence of chronic inflammation. Fibrosis develops in a tissue space by organization of the inflammatory exudate occupying the tissue space.

Q. Classify different types of cells.

Ans. Cells are divided into three groups based on their ability to repair themselves:

1. **Labile/continuously dividing cells:** Cells which replenish their damaged/injured counterparts by continuous division and maturation of cells from the stem cell pool, eg, surface epithelial cells (skin, oral cavity, vagina and cervix), haematopoietic cells in bone marrow and columnar epithelium of GIT.
2. **Stable/quiescent cells (facultative mitotic cells):** Cells of this type are in G_0 stage of cell cycle, and do not replicate actively in their normal state; however, they are capable of proliferating in response to loss of tissue mass, eg, parenchymal cells of solid organs (liver, kidneys and pancreas), endothelial cells, fibroblasts, smooth muscle cells, chondrocytes and osteocytes.
3. **Permanent/nondividing cells:** Terminally differentiated cells, which are nonproliferative and incapable of regenerating, eg, neurons, skeletal muscle and cardiac muscle cells.

Q. What are stem cells?

Ans. Stem cells are cells with **self-renewal capacity**. They are characterized by **asymmetric replication** (a property of stem cells by virtue of which, after every cell cycle, some of the daughter cells retain their self-renewal capacity while the others enter a differentiation pathway, and are converted into a mature nondividing population). Stem cells may be

- **Embryonic stem cells**: Stem cells that are isolated from embryos are called **embryonic stem cell (ES**; Fig. 3.1). These are the most undifferentiated stem cells located in the inner cell mass of the blastocyst. They can give rise to any type of cell in the body and are therefore also called **totipotent stem cells**.
- **Adult stem cells** have a markedly restricted differentiation capacity and are usually lineage specific. Adult stem cells located outside the bone marrow and in the tissue are

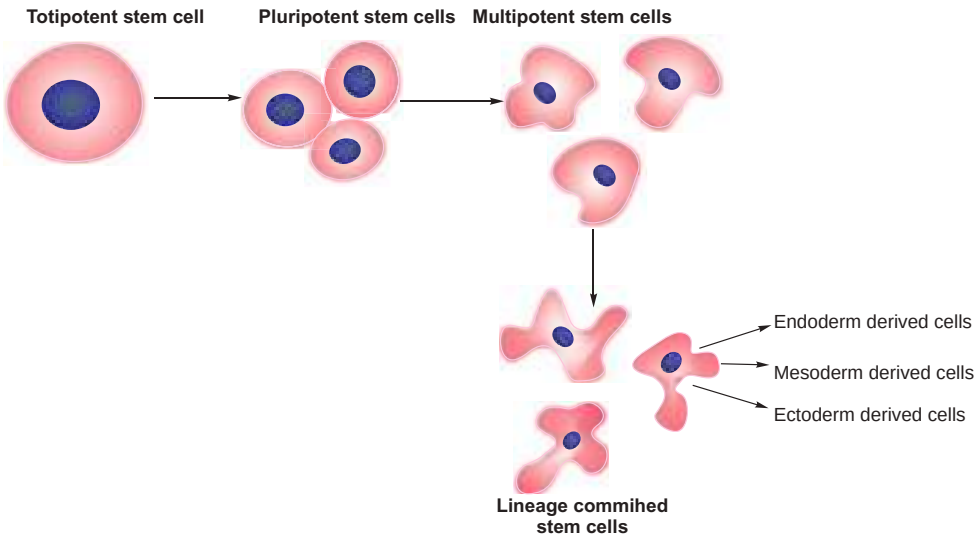


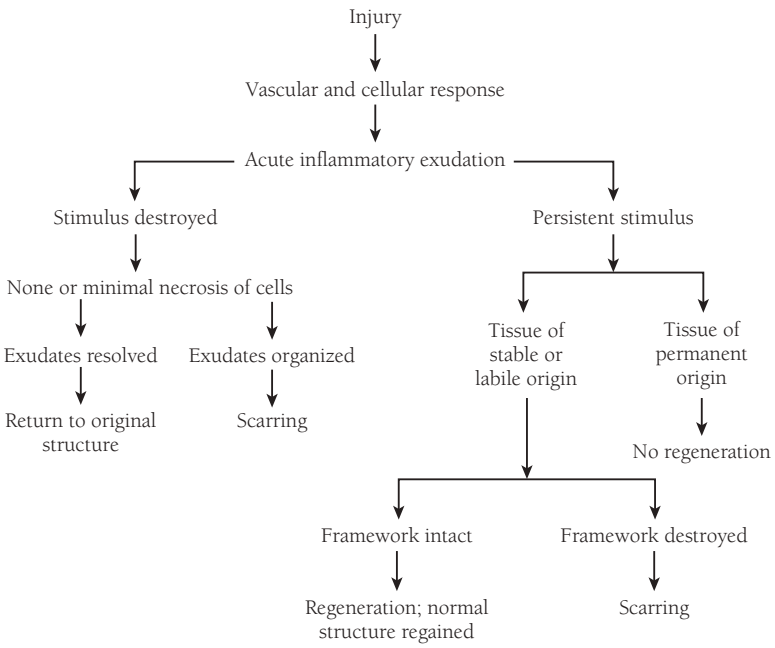
FIGURE 3.1. Embryonic stem cells.

generally referred to as **tissue stem cells**, eg, liver stem cells which differentiate into hepatocytes and biliary cells. Tissue stem cells are located within a protected microenvironment called '**stem cell niches'.** Neural tissue stem cells are located in the subventricular area and dentate gyrus; whereas, skin stem cells are found in the hair follicle bulge and corneal stem cells are present in the limbus.

The most elaborately studied stem cells are **haematopoietic stem cells** as well as **stromal cells located in the bone marrow**. The former are capable of differentiating into various blood cell lineages, while the latter, also called mesenchymal stem cells, are multipotent and can differentiate into a variety of stromal cells (chondrocytes, osteocytes, adipocytes and myocytes). Haematopoietic stem cells can be clinically used to replace depleted marrow cells (following chemotherapy for leukaemia) or provide normal cells to overcome red cell defects (sickle cell disease). Marrow stromal cells (mesenchymal stem cells) can be used clinically to provide stromal cellular scaffolding for tissue regeneration.

Q. Enumerate the sequence of events involved in reparative response following an injury.

Ans. Pathways of reparative response following an injury (Flowchart 3.1):



FLOWCHART 3.1. Pathways of reparative response following injury.

Q. Write briefly on cell cycle.

Ans. During differentiation, mammalian cells alternate between a phase of division (**mitosis**) and a resting phase (**interphase**). The sequence of events that control DNA replication and mitosis and, hence, cellular proliferation is labelled cell cycle (Fig. 3.2).

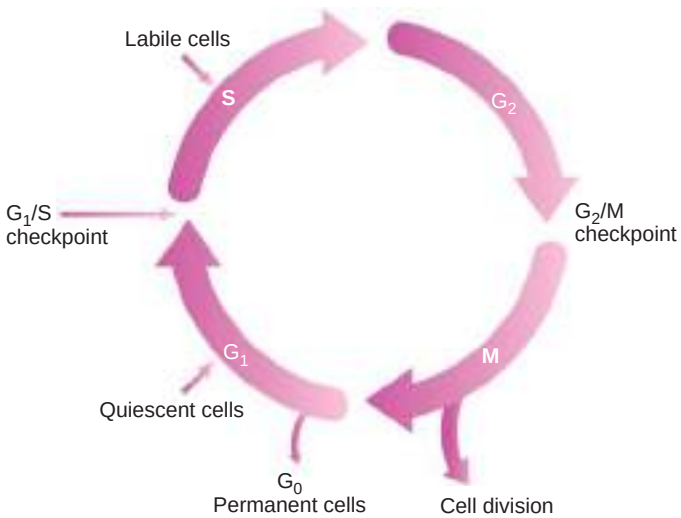


FIGURE 3.2. Cell cycle.

Phases of Cell Cycle

Interphase is supposedly a resting stage between cell divisions; however, it is actually a period of several essential activities which are a prerequisite for making the next mitosis possible, eg, synthesis of RNA and protein production. Interphase lasts about 12–24 hours in mammalian tissue and can be divided into four phases: Gap 0 (G_0), Gap 1 (G_1), S (synthesis phase) and Gap 2 (G_2). Human genome is doubled in the **S phase**, and **halved** during **M phase**. The period between M and S phase is called **G_1**; and that between S and M phases is called **G_2**. So, the cell cycle consists of:

- **G_0 (resting phase)**: When a cell leaves the cell cycle and quits dividing, it is said to be in G_0 phase. This may be a transient phase or indefinite as in the case of a permanent cell that has reached the end stage of its development and will not divide (eg, cardiac myocytes or neurons).
- **G_1 (presynthetic growth phase)**: In this phase, cells enlarge due to production of RNA and synthesis of proteins. G_1 checkpoint is an important controlling mechanism that ensures adequate preparation for DNA synthesis.
- **S (synthetic phase)**: Synthesis of DNA and duplication of the centrosome takes place in this phase.
- **G_2 (premitotic growth phase)**: The cell continues to grow and produce new proteins like G_1 phase. Another checkpoint (G_2 checkpoint) towards the end of G_2 phase determines whether the cell is adequately prepared to proceed to the M phase and divide or not.
- **M phase (mitotic phase)**: After protein synthesis and enlargement, the cell enters a phase of division to give rise to two similar daughter cells. Mitosis lasts only 1–2 hours. As in both G_1 and G_2, the mitotic phase also has a checkpoint (called metaphase checkpoint) that ensures the readiness of the cell to complete cell division.

Control of the Cell Cycle

Progression of the cell cycle is tightly regulated by the following proteins:

- **Cyclins:** These are classified as *G1 cyclins* (D cyclins), *S-phase cyclins* (cyclins E and A) and *mitotic cyclins* (B cyclins). Their levels vary corresponding to the different phases of cell cycle (cyclins are named so because of the cyclical nature of their production and degradation during cell cycle).
- **Cyclin-dependent kinases (CDKs):** These cyclin-associated enzymes include a G_1 CDK (CDK4), an S-phase CDK (CDK2) and an M-phase CDK (CDK1). Their cellular levels are relatively stable and they get activated on binding to the appropriate cyclin.

Steps in the Cell Cycle

1. The binding of G_1-cyclins to CDKs is an indication to the cell to initiate chromosomal replication. CDKs activated by combining with cyclins initiate the cell cycle by phosphorylating proteins such as retinoblastoma (RB) susceptibility protein, which normally prevents cells from replicating by forming a tight, inactive complex with the transcription factor E2F. Phosphorylation releases RB which activates E2F, which in turn stimulates transcription.
2. The cyclin–CDK complexes are tightly regulated by *CDK inhibitors (CDKIs)*, which themselves are inhibited by other growth factors. CDKIs include several families. One family comprised of p21 (CDKN1A), p27 (CDKN1B) and p57 (CDKN1C) inhibits multiple CDKs. Another family comprised of p15 (CDKN2B), p16 (CDKN2A), p18 (CDKN2C) and p19 (CDKN2D) has selective effects on cyclins CDK4 and CDK6.
3. The G_1/S checkpoint ensures the integrity of DNA before replication; whereas, the G_2/M checkpoint does the same after replication. These checkpoints monitor whether the cell is prepared enough to enter mitosis. When there is DNA damage, the activation of checkpoints delays the cell cycle and triggers DNA repair mechanisms. If DNA damage is too severe to be repaired, the cells are eliminated by apoptosis.

4. Increased levels of cyclin A bound to CDK2 initiates DNA duplication in the nucleus.
5. The levels of mitotic or B cyclins rise in the M phase and the M-phase promoting factor (the complex of mitotic B cyclins with the M-phase CDK or CDK1) initiates the formation of the mitotic spindle, condensation of the chromatin as well as dissolution of the nuclear envelope.

Q. Enumerate the growth factors and cytokines involved in regeneration and wound healing.

Ans. Growth factors (GFs) are mostly proteins that prolong survival and induce proliferation of specific cells (Table 3.1). They bind to specific receptors and deliver signals that stimulate expression of genes whose products induce growth. They have the following functions:

1. Cell cycle activation (by direct stimulation or removal of blocks that inhibit cell cycle)
2. Prevention of apoptosis
3. Enhanced synthesis of cellular proteins

TABLE 3.1. Growth factors and cytokines involved in regeneration and wound healing

Type of growth factor	Symbol	Receptor	Sources	Functions
Epidermal growth factor	EGF	EGFR1 (ERBB1)	Platelets, macrophages, salivary glands, keratinocytes, etc.	Mitogenic for keratinocytes and fibroblasts; stimulates keratinocyte migration and granulation tissue formation
Fibroblast growth factor (FGF) family	FGF	FGFRs (1-4)	Platelets and macrophages	Wound repair. angiogenesis
Transforming growth factor-α	TGF-α	ERB B2 (HER-2 or HER-2/Neu)	Activated macrophages, T lymphocytes, keratinocytes, etc.	Similar to EGF; stimulates replication of hepatocytes and many epithelial cells
Hepatocyte growth factor/scatter factor	HGF/SF	c-MET	Mesenchymal cells	Enhances proliferation of epithelial and endothelial cells
Vascular endothelial cell growth factor (isoforms A, B, C and D)	VEGF	VEGFR-1, VEGFR-2 and VEGFR-3	Mesenchymal cells	Increases vascular permeability; mitogenic for endothelial cells
Platelet-derived growth factor (isoforms A, B, C and D)	PDGF	PDGFR α and β	Platelets, macrophages, endothelial cells, keratinocytes, smooth muscle cells	Chemotactic for neutrophils, macrophages and smooth muscle cells; stimulates production of matrix metalloproteinases (MMPs), fibronectin, stimulates angiogenesis
Tumour necrosis factor	TNF	TNF-R	Macrophages, mast cells, T lymphocytes	Activates macrophages; regulates other cytokines
Interleukins	ILs	IL-R	Macrophages, mast cells, lymphocytes and many tissues	Chemotactic, angiogenic; regulate other cytokines
Transforming growth factor-β (TGF-β isoforms - TGF-β1, TGF-β2 and TGF-β3)	TGF-β	TGF-β receptors (types I and II)	Platelets, T lymphocytes, macrophages, endothelial cells	Chemotactic, angiogenic, mitogenic for fibroblasts, stimulates wound contraction, and matrix deposition
Interferons	IFN-α	Interferon receptors	Lymphocytes and fibroblasts	Activate macrophages, inhibit fibroblast proliferation, regulate other cytokines

Q. What are the different signalling mechanisms in cell growth? Write briefly on receptors and signalling pathways involved in healing and repair.

Ans. Signalling Mechanisms in Cell Growth

Growth factors (GFs) act by binding to specific receptors, which deliver signals to target cells. Signalling may be:

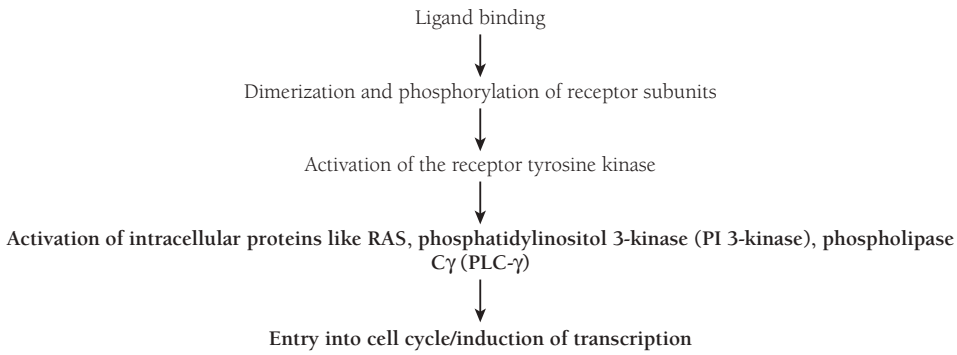
1. Autocrine (GFs act on the same cells that secrete them, eg, HGF/SF)
2. Paracrine (GFs act on cells adjacent to the cells that secrete them, eg, produced by macrophages and action on fibroblasts)
3. Endocrine (produced by endocrine cells and carried in the blood stream to distant target cells, eg, hormones)

Receptors and Signalling Pathways

- Binding of a ligand to its receptor triggers a series of intracellular signals that induce transcription factor activation or repression leading to different cellular events.
- Receptors can be on the surface of the target cells, in the cytoplasm or in the nucleus.

Signal transduction can originate from three types of receptors:

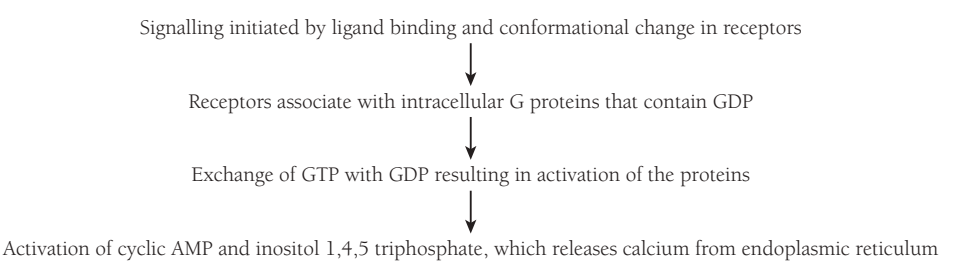
1. **Receptors with intrinsic tyrosine kinase activity** (Flowchart 3.2)**:** These are dimeric transmembrane molecules having
   (a) An extracellular ligand-binding domain.
   (b) Transmembrane region.
   (c) A cytoplasmic tail with tyrosine kinase activity.



FLOWCHART 3.2. Signal transduction mediated by receptors with intrinsic tyrosine kinase activity.

Example: An important pathway stimulated by Rous sarcoma virus (RAS) activation is the mitogen-activated protein (MAP) kinase cascade, which is involved in the intracellular signalling of many growth factors, eg, Insulin, EGF, TGF-α, HGF, PDGF and VEGF.

2. **Seven transmembrane G protein–coupled receptors** (Flowchart 3.3)**:** Polypeptides containing seven transmembrane α-helical segments (traverse the plasma membrane 7 times). *Examples*: Vasopressin, histamine, serotonin, glucagon and chemokines.



FLOWCHART 3.3. Signal transduction mediated by seven transmembrane G protein–coupled receptors.

3. **Receptors lacking intrinsic tyrosine kinase activity**
   Receptors can transmit extracellular signals to nucleus by activating Janus kinases (JAKs) which activate cytoplasmic transcription factors STATs (signal transducers and activators of transcription), which in turn enter the nucleus and activate gene transcription.
   Examples: Receptors for cytokines like IL-2, IL-3, interferons, granulocyte monocyte-colony stimulating factor (GM-CSF) and growth hormone.
   Note: All ligands do not induce stimulatory signals, growth inhibitory signals are also generated, eg, TGF-β binding with its receptor phosphorylates some intracellular proteins, which in turn increase the synthesis of CDK inhibitors and block the activity of transcription factors and cell cycle progression.
4. **Nuclear receptors**
   Lipid soluble ligands (steroid hormones, thyroid hormone, vitamin D and retinoids) can diffuse into the cell to interact with intracellular proteins forming a receptor–ligand complex which in turn binds to the inactive receptor located in the nucleus to activate it. Activated receptor binds to the specific DNA sequences known as hormone response elements on target genes or transcription factor.
   Example: A group of receptors called peroxisome proliferator-activated receptors (PPARs) involved in inflammation and atherosclerosis.

Q. Write briefly on extracellular matrix (ECM) and cell-matrix interactions.

Ans. Tissue repair depends on

1. Growth factor activity
2. Interaction between cells and ECM components

ECM is a constantly changing macromolecular complex, which assembles into a network that surrounds and supports the cells. It has the following functions:

1. Provides support and anchorage for cells, segregates tissues from one another and regulates intercellular communication.
2. Sequesters growth factors and serves as a reservoir for them (FGF and HGF; allows rapid deployment of growth factors after injury for regeneration).
3. Provides a substrate for cell adhesion.
4. Sequesters water to provide turgor to soft tissues.
5. Sequesters minerals to provide rigidity to bone.
6. Regulates proliferation and controls cell growth, movement and differentiation (by signalling through cellular receptors of integrin family).

ECM Exists in Two Different Forms

1. **Interstitial matrix:** Synthesized by mesenchymal cells, this randomly fills up the space between cells and supporting vascular and smooth muscle structures.
2. **Basement membrane:** The interstitial matrix organizes itself around epithelial, endothelial and smooth muscle cells to form a meshwork, which anchors down the above cells to loose connective tissue underneath. This meshwork is called 'basement membrane'. Its major components are amorphous nonfibrillar type IV collagen and laminin.

Components of ECM

- **Fibrous structural proteins** such as collagens and elastins for tensile strength and recoil
- **Water hydrated gels** such as proteoglycans and hyaluronate for resilience and lubrication
- **Adhesive glycoproteins** that connect the matrix elements to one another and to cells

Fibrous Structural Proteins

1. **Fibrillar and nonfibrillar collagen**
   (a) Collagen provides the extracellular framework of most tissues in the body. All collagen types have a triple helical structure. Three separate polypeptide chains are braided into a rope-like triple helix.
   (b) Collagen proteins are rich in hydroxyproline and hydroxylysine.
   (c) Thirty different types are known.
   (d) Some types (I, II, III and V) form fibrils by virtue of lateral cross-linking of the triple helices.
   (e) Cross-linking is the result of covalent bonding catalysed by the enzyme lysyl oxidase in the presence of vitamin C.
   (f) Nonfibrillar collagens form the basement membrane (type IV collagen) or are components of structures like intervertebral discs (type IX collagen) or dermoepidermal junction (type VII collagen).
2. **Elastin**
   (a) Gives elasticity to tissues, allowing them to stretch when needed and then return to their original state.
   (b) Especially important in walls of large vessels, lungs, uterus, skin and ligaments.
   (c) Elastins are synthesized by fibroblasts and smooth muscle cells.
   (d) Morphologically, consist of a central core of elastin surrounded by a meshwork of a fibrillar glycoprotein.

Water Hydrated Gels

(a) Provide compressibility to the tissue and serve as reservoirs of growth factors.
(b) Proteoglycans are comprised of long polysaccharides called glycosaminoglycans (heparan sulphate and dermatan sulphate) linked to a protein backbone.
(c) Hyaluronan is comprised of disaccharide repeats without any protein core.

Adhesive Glycoproteins and Adhesion Receptors

Structurally, diverse molecules that are involved in cell-to-cell adhesion, linkage between cells and ECM and binding between ECM components include

(i) **Fibronectin** (main constituent of interstitial matrix) is a protein that connects cells with collagen fibres in the ECM, allowing cells to move through the ECM. Fibronectins bind collagen and cell surface integrins, facilitating cell movement.
(ii) **Laminin** (main component of basement membrane) assists in cell adhesion and binds cells to other ECM components such as type IV collagen and heparin sulphate.
(iii) **Adhesion receptors** are also known as **cell adhesion molecules or CAMs**. CAMs are grouped into four categories, namely, immunoglobulins, cadherins, selectins and integrins.

Q. Write briefly on cell and tissue regeneration.

Ans. The ability to regenerate is dependent on the type of cell.

- **Labile tissues** undergo continuous renewal.
- In **stable tissues,** the tissue regeneration occurs but is a limited process (with the exception of liver). Pancreas, adrenals, kidneys, thyroid and lungs have limited regenerative capacity; however, as much as 40–60% of the liver may regenerate subsequent to its loss. Regeneration is dependent on many variables, eg, growth factors, inhibitors, signal transduction pathways, transcription factors and ECM proteins.
- TNF and IL-6 stimulate transition of the cells from G_0 to G_1 phase of cell cycle and HGF and EGF family of factors help in progression through the rest of the cell cycle. FGF and TGF-α are mitogenic for hepatocytes. Most epithelial cells share a common receptor (EGFR—epidermal growth factor receptor) with intrinsic tyrosine kinase activity.

Q. Write briefly on repair by connective tissue.

Ans.

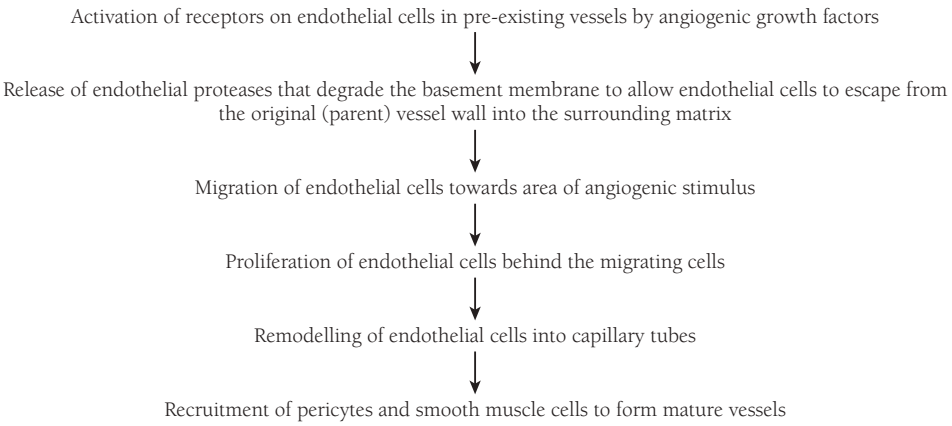
- Repair by connective tissue occurs when:
  - There is severe injury so that parenchymal tissue as well as connective tissue framework of the organ is destroyed.
  - Nondividing cells are injured.
- Repair begins within 24 hours; by 3–7 days, a special type of tissue called granulation tissue (so-called because of the pink, soft and granular-gross appearance) is formed.
- Repair by connective tissue deposition consists of four main stages:
  1. Formation of new blood vessels (angiogenesis)
  2. Migration and proliferation of fibroblasts
  3. Deposition of ECM
  4. Remodelling of fibrous tissue

Q. Write briefly on angiogenesis.

Ans. Two processes assemble blood vessels (Fig. 3.3):

1. **Vasculogenesis:** Involves formation of primitive vascular structures from angioblasts (endothelial cell precursors) in a manner resembling embryonal development of the vascular system.
2. **Angiogenesis:** Neovascularization in which pre-existing vessels send out capillary sprouts to produce new vessels with or without pericytes and smooth muscle cells (mostly seen during repair of damaged tissue).

Steps in angiogenesis are shown in Flowchart 3.4**.**



FLOWCHART 3.4. Steps in angiogenesis.

Structural ECM proteins: Participate in the process of vessel sprouting through interactions with integrin receptors.
Nonstructural proteins: Contribute to angiogenesis by destabilizing cell–ECM interactions to facilitate continued cell migration or degrade the ECM to permit remodelling and in growth of vessels.

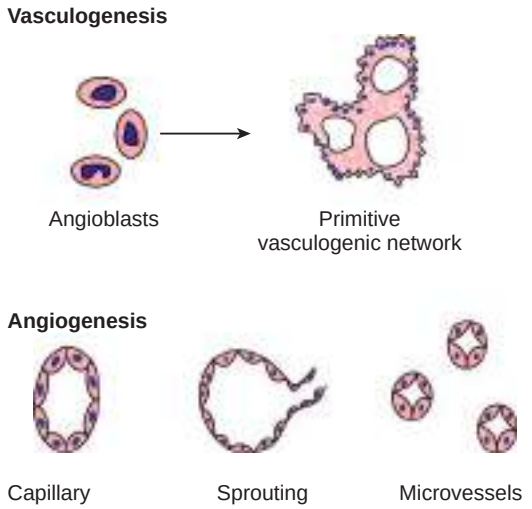


FIGURE 3.3. Mechanism of formation of new blood vessels.

Growth Factors Involved in Angiogenesis

1. **VEGFs**
   (a) Family of growth factors that include VEGF isoforms–A, B, C, D
   (b) Hypoxia, PDGF, TGF-β and TGF-α induce release of VEGFs
   (c) VEGFs bind to a family of receptors with tyrosine kinase activity and stimulate both proliferation and motility of endothelial cells
2. **FGFs**
   (a) Family with more than 20 members
   (b) Best characterized are FGF1 (acidic FGF) and FGF2 (basic FGF)
   (c) FGFs bind to a family of receptors with tyrosine kinase activity and stimulate proliferation of endothelial cells and promote migration of macrophages and fibroblasts to the damaged area

Q. Write briefly on scar formation.

Ans. There are two major steps in scar formation:

1. Migration of fibroblasts to the damaged area followed by proliferation.
2. Deposition of ECM by the same cells. Deposition of ECM involves the following:
   (a) Recruitment and stimulation of fibroblasts which is driven by many growth factors, eg, PDGF, FGF2, TGF-β (elaborated by endothelium and macrophages).
   (b) Macrophages clear extracellular debris and fibrin and also elaborate a host of mediators that induce fibroblast proliferation and ECM production.
   (c) As healing progresses, the number of proliferating fibroblasts and new vessels decrease and collagen synthesis and ECM deposition increases.
   (d) **Decreased collagen degradation rather than increased collagen synthesis is responsible for net collagen accumulation.**
   (e) Ultimately, the granulation tissue scaffold is converted into scar tissue composed of fibroblasts, dense collagen, fragments of elastic tissue and other ECM components.

Q. Write briefly on healing by primary or first intention.

Ans. Primary union is seen in incised wounds with opposed edges (clean and uninfected wound). The steps in healing by primary intention are summarized in Flowchart 3.5 and Fig. 3.4A.

Wound

↓

- Filled with blood clot and inflammatory cells
- Neutrophils appear at the margin of the wound

} Day 1

↓

- Neutrophils largely replaced by macrophages
- Granulation tissue formation begins and infiltrates the incision space
- Collagen fibres form at the margins of the incision and are laid down vertically
- Thickening of the epithelial layer due to epithelial cell proliferation and migration of epithelial cells along incised margins

} Day 3

↓

- Incisional space is filled with granulation tissue
- Neovascularization is at its peak
- Collagen fibres become abundant and bridge the incisional gap
- Epidermis regains its normal thickness
- Differentiation of surface cells yields mature epidermal architecture

} Day 5

↓

- Continued accumulation of collagen and proliferation of fibroblasts
- Regression of vessels and blanching

} Day 7

↓

- Scar tissue formation by the end of the first month
- Gradual gain in tensile strength overtime

FLOWCHART 3.5. Steps in healing by primary intention.

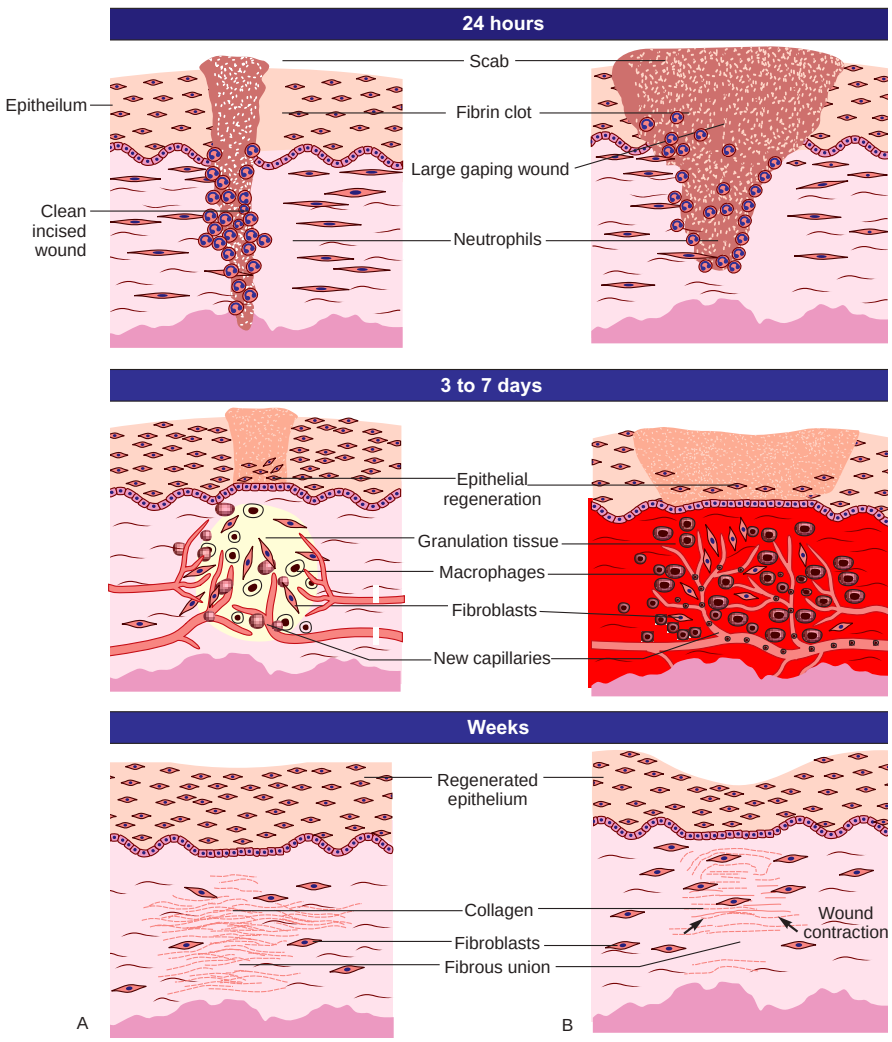


FIGURE 3.4 A and B. Healing by (A) primary and (B) secondary intentions.

Q. Write briefly on healing by secondary intention.

Ans. Secondary union (Fig. 3.4B) is seen in open wounds with separated edges, extensive loss of cells and large defects.

Characteristic Features of Healing by Secondary Intention

- Associated with large defects filled with blood clots, necrotic debris and exudate.
- Inflammatory reaction is more intense.
- Large amounts of granulation tissue are deposited.
- There is formation of epithelial spurs from margins of the wound.
- Typically demonstrates 'wound contraction' which is mediated by myofibroblasts and aids in decreasing the gap between the dermal edges of the large wound.
- Substantial scar formation and thinning of the epidermis is seen.

Regaining Wound Strength

- After 7–10 days, 10% of the original tensile strength is regained.
- After 3 months, 80% of the original tensile strength is regained.

Q. Differentiate between healing by primary and secondary intention.

Ans. Differences between healing by primary and secondary intention are enlisted in Table 3.2.

TABLE 3.2. Differences between healing by primary and secondary intention

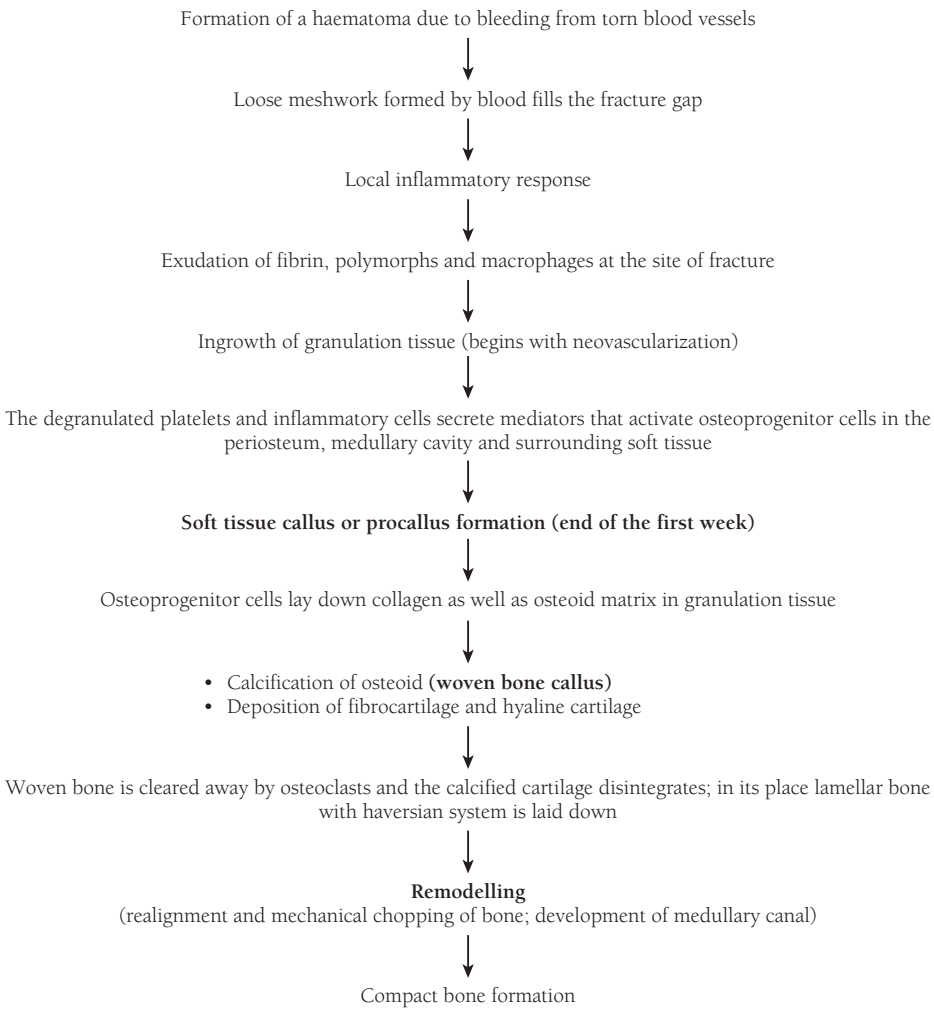
Features	Healing by primary intention	Healing by secondary intention
Nature of wound	Seen in incised wounds with well opposed edges (clean and uninfected wound)	Seen in large, open, infected wounds with separated edges; associated with extensive loss of cells
Amount of fibrin and blood	Filled with moderate amount of fibrin and blood	Filled with a large blood clot and necrotic debris and exudate
Inflammatory reaction	Less intense	More intense
Amount of granulation tissue	Less granulation tissue	Extensive granulation tissue
Wound contraction	Wound contraction is not seen	Wound contraction is seen
Complications	Less common	More common

Q. Write in detail on healing in specialized tissues.

Ans. Healing in Specialized Tissues

1. **Fracture healing**
   Fractures can be:
   (a) Traumatic or pathological (due to a pre-existing disease)
   (b) Complete or incomplete
   (c) Simple (overlying tissue is intact), comminuted (bone is splintered or displaced) or compound (fracture site communicates with the skin surface)
   (d) Stress fracture (slowly developed fracture, which develops over a period of increased physical activity)

There are three main steps in callus formation (Flowchart 3.6):
- Procallus formation
- Osseous callus formation
- Remodelling



FLOWCHART 3.6. Steps in callus formation.

2. **Healing of nervous tissue:**
   (a) **Central nervous system:** Nerve cells of brain, spinal cord and ganglia once destroyed are not replaced. Neuroglial cells, however, may show proliferation called **gliosis**.
   (b) **Peripheral nervous system:** Proliferation of Schwann cells and fibrils from distal ends is seen in response to injury.
3. **Healing of muscle:**
   (a) **Skeletal muscle:**
      (i) If the muscle sheath is intact, sarcolemmal tubes appear along endomysium and restore muscle fibres, eg, Zenker degeneration of the muscle in typhoid.
      (ii) If the muscle sheath is damaged, a disorganized multinucleated mass and scar comprised of fibrovascular tissue form, eg, Volkmann ischaemic contracture.
   (b) **Cardiac muscle:** Replaced by the permanent scar tissue, eg, cardiac muscle is replaced by fibrous tissue in myocardial infarction
4. **Healing of solid epithelial organs:**
   In parenchymal cell damage with intact basement membrane, regeneration and restoration are possible; however, gross tissue damage to these organs lead to healing by fibrous scarring, eg, chronic pyelonephritis.

Q. Enumerate the complications of fracture healing.

Ans. Complications of Fracture Healing:

1. Fibrous union (inadequate immobilization permits constant movement at the fracture site so that the normal constituents of callus do not form; callus is comprised of only fibrous tissue and cartilage)
2. Nonunion
3. Delayed union
4. Pseudoarthrosis (if a nonunion allows too much motion along the fracture gap; the central portion of the callus undergoes cystic degeneration and its luminal surface becomes lined by synovial-like cells creating a false joint called pseudoarthrosis)

Q. Enumerate the factors that retard wound healing.

Ans. Factors that retard wound healing may be:

1. ***Local***
   (a) Decreased blood supply
   (b) Denervation
   (c) Local infection
   (d) Foreign body
   (e) Mechanical stress
   (f) Large amounts of haemorrhage and necrosis
2. ***Systemic***
   (a) Old age
   (b) Malnutrition
   (c) Anaemia
   (d) Obesity
   (e) Drugs (steroids)
   (f) Systemic infection
   (g) Genetic disorders, eg, Marfan syndrome and Ehlers–Danlos disease
   (h) Diabetes mellitus
   (i) Uraemia
   (j) Vitamin and trace metal (zinc and copper) deficiency

Q. Enumerate the complications of wound healing.

Ans. Complications of wound healing are:

1. Deficient scar formation leading to wound dehiscence (rupture) or ulceration
2. Formation of exuberant granulation tissue which protrudes above the level of the surrounding skin and blocks re-epithelialization (proud flesh)
3. Excessive formation of repair components, eg, collagen leading to hypertrophied scar or keloid formation
4. Development of contractures (palmar or Dupuytren contracture and plantar contracture)
5. Development of incisional hernia, neoplasia, pigmentation or implantation cysts

4

Haemodynamic Disorders, Thrombosis and Shock

Q. Define oedema. Write briefly on its types.

Ans. Oedema is defined as abnormal and excessive accumulation of fluid in interstitial tissue spaces and serous cavities.

Approximately, 60% of body weight is water—two-thirds of which is intracellular and one-third extracellular. The extracellular space is divided into interstitial and intravascular compartments. Bulk of the extracellular water is formed by interstitial fluid and only 5% of the body's water is present as blood plasma. If the net influx of fluid exceeds the lymphatic drainage, the excessive volume of fluid may accumulate either within the interstitial matrix (**interstitial oedema**) or in the serous body cavities (**effusion**).

Examples of Oedema/Effusion

- Periorbital oedema
- Dependent oedema
- Generalized oedema or anasarca
- Hydrothorax or pleural effusion
- Hydropericardium or pericardial effusion
- Hydroperitoneum or ascites

Oedema causes a palpable swelling and may be the result of either too much pressure or too little protein within the blood vessels.

Classification of Oedema

- ***Localized*** or ***generalized oedema***, based on the distribution and extent of involvement. Localized oedema is limited to a small area, eg, an organ (organ–specific oedema) or a limb (elephantiasis, oedema due to venous obstruction as seen in deep vein thrombosis, allergic laryngeal oedema as seen in anaphylaxis and localized inflammatory oedema). Generalized oedema, on the other hand, may involve the entire body (oedema due to congestive cardiac failure, nephrotic syndrome and nutritional deficiency).
- ***Transudative*** or ***exudative oedema/effusion***, based on the composition of the fluid. The differences between transudative and exudative effusion are summarized in Table 4.1.

Consequences of Oedema

Oedema may compromise cellular function in the following ways:

- Due to expansion of the interstitial space, there is an increase in the diffusion distance for oxygen and other nutrients, which hampers cellular metabolism, eg, impaired gas exchange due to pulmonary oedema.
- Expansion of the interstitial space also interferes with the removal of toxic by-products of cellular metabolism.

Q. Enumerate the differences between exudate and transudate.

Ans. Differences between exudate and transudate are tabulated in Table 4.1.

TABLE 4.1. Differences between exudate and transudate

Features	Exudate	Transudate
Definition	Oedema associated with increased vascular permeability	Filtrate of blood or plasma; no increase in vascular permeability observed
Nature	Inflammatory oedema	Noninflammatory oedema
Protein content	1. High (more than 4 g/dl) 2. Has high fibrinogen and tendency to coagulate	1. Low (less than 3 g/dl) 2. Mainly albumin, low fibrinogen
Specific gravity	High (more than 1.018)	Low (less than 1.015)
pH	>7.3	<7.3
LDH	High Fluid LDH/serum LDH ratio is >0.6	Low Fluid LDH/serum LDH ratio is <0.6
Cells	Highly cellular; rich in polymorphs	Few, mainly mesothelial cells
Example	Pus seen in pyogenic infections	Fluid in congestive cardiac failure

Q. What are the factors affecting fluid balance across capillaries?

Ans. Factors affecting fluid balance across capillaries (Fig. 4.1) can be summarized as follows:

(a) Plasma colloid oncotic pressure (which tends to drive water and salts into the vessels)
(b) Capillary hydrostatic pressure (which tends to drive water and salts out of the vessels into the interstitial space)
(c) Lymphatic drainage (which tends to drain the interstitial space)
(d) Sodium balance (sodium retention increases hydrostatic pressure and causes a dilutional decrease in the colloid osmotic pressure)

- Oedema occurs when there are
  (a) Abnormalities in the hydrostatic and oncotic pressures acting across the vessel walls
  (b) Alterations in the endothelial wall structure
  (c) Alterations in the lymphatic outflow system
- Normally, at the arteriolar end of the capillary bed, the hydrostatic pressure exceeds the plasma oncotic (colloid osmotic) pressure pushing the water and electrolytes out from the vessels into the interstitial tissue. At the venous end of the capillary bed, osmotic pressure is more than the hydrostatic pressure and hence the fluid is reabsorbed from the interstitial tissue into the vessels at this end. The interstitial fluid is drained by the lymph vessels and this eventually returns to the veins via the thoracic duct.

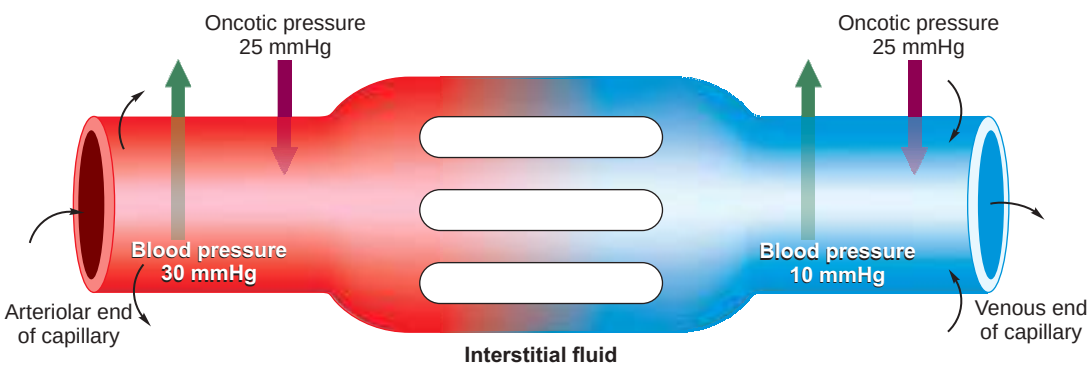


FIGURE 4.1. Factors affecting fluid balance across capillaries.

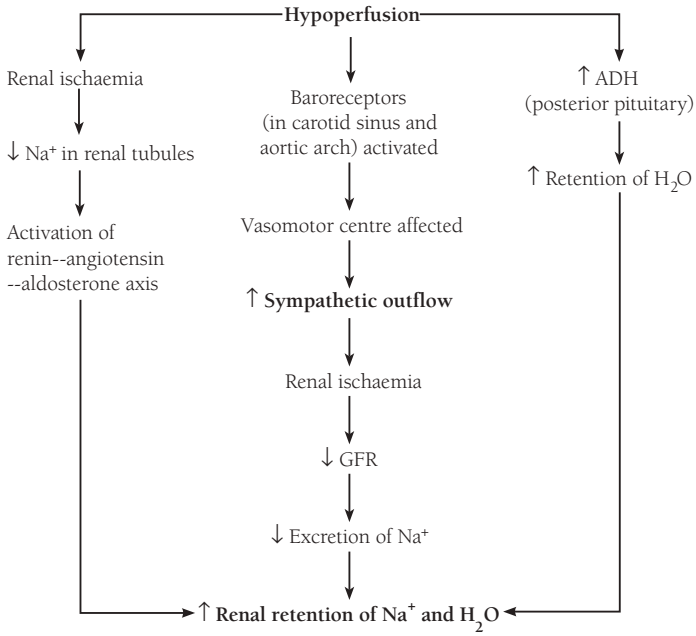
Q. What are the pathophysiologic categories of oedema?

Ans. Important pathophysiologic categories of oedema are enumerated below:

1. **Increased hydrostatic pressure**
   (a) Hydrostatic pressure of capillaries is the force that tends to drive fluid through capillary wall into interstitial space.
   (b) A rise in hydrostatic pressure at venular end of a capillary to a level more than plasma oncotic pressure results in oedema.
   (c) Increased hydrostatic pressure may be due to
      (i) Impaired venous return
         - Congestive heart failure
         - Constrictive pericarditis
         - Ascites (liver cirrhosis)
         - Venous obstruction
         - Thrombosis
         - External pressure due to a mass
      (ii) Arteriolar dilatation
         - Heat
         - Neurohumoral dysregulation
2. **Reduced plasma oncotic pressure**
   (a) Normally, plasma oncotic pressure is exerted by plasma proteins and drives fluid into the vessels.
   (b) Reduced oncotic pressure causes increased movement of fluid into interstitial space.
   (c) Decreased plasma oncotic pressure may be due to
      (i) Protein-losing glomerulopathies (nephrotic syndrome)
      (ii) Reduced synthesis of proteins (liver cirrhosis)
      (iii) Decreased intake of proteins (malnutrition as in famine oedema)
      (iv) Protein-losing gastroenteropathies and malabsorption
3. **Lymphatic obstruction**
   (a) Normally, interstitial fluid escapes via lymphatic system. Lymphatic obstruction causes accumulation of fluid in interstitial space.
   (b) Lymphatic obstruction may be
      (i) Inflammatory
      (ii) Neoplastic
      (iii) Post-surgical
      (iv) Post-irradiation
      (v) Due to congenital absence of lymphatics
4. **Sodium retention:** Increased sodium retention is invariably associated with water retention, which leads to increased plasma volume as well as hydrostatic pressure. Dilutional effect on albumin leads to decreased plasma colloid oncotic pressure. Causes of sodium retention include
   (a) Excessive salt intake with renal insufficiency
   (b) Increased tubular reabsorption of sodium
   (c) Renal hypoperfusion
   (d) Increased renin–angiotensin–aldosterone secretion
5. **Inflammation:** Endothelial lining may be injured by toxins and their products, eg, histamine, anoxia, venoms, drugs and chemicals. Endothelial damage leads to increased vascular permeability and leakage of plasma proteins into the interstitial tissue resulting in oedema.

Q. Write briefly on normal regulatory mechanisms responsible for maintaining sodium and water balance.

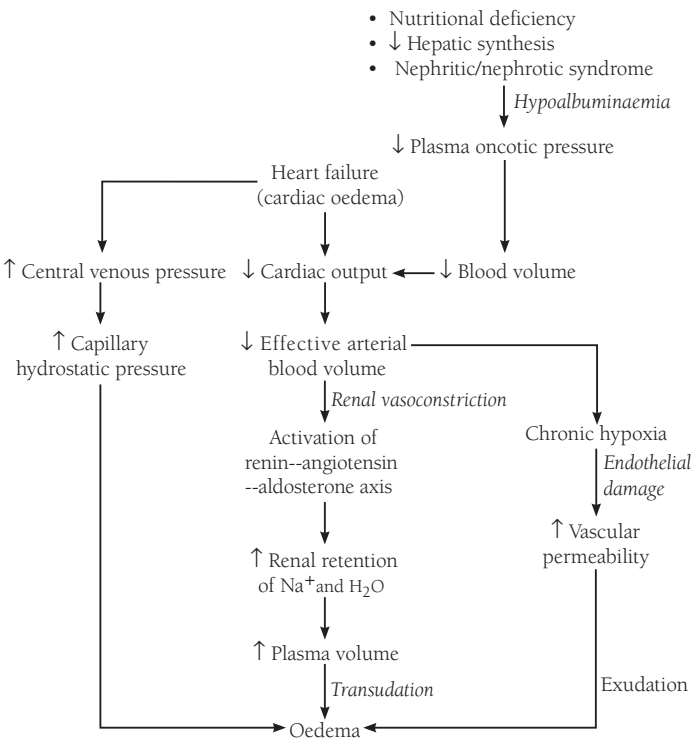
Ans. Eighty percent of sodium is reabsorbed by proximal convoluted tubule under the influence of intrinsic and extrinsic renal mechanisms (Flowchart 4.1).



FLOWCHART 4.1. Normal regulatory mechanisms responsible for maintaining sodium and water balance.

Q. Write briefly on the pathogenesis of oedema.

Ans. Pathogenesis of oedema is shown in Flowchart 4.2.



FLOWCHART 4.2. Pathogenesis of oedema.

Q. Write briefly on renal oedema.

Ans. Oedema due to renal dysfunction typically appears first in loose connective tissue, eg, the eyelids (periorbital oedema).

- Causes of renal oedema include **nephrotic syndrome, glomerulonephritis** (acute glomerulonephritis, rapidly progressive glomerulonephritis) and **acute tubular injury**. Nephrotic syndrome is characterized by persistent and heavy proteinuria causing a reduced plasma oncotic pressure leading to generalized severe oedema.
- Also, a reduction in the plasma volume causes activation of the renin–angiotensin–aldosterone mechanism, thereby causing retention of sodium and water or oedema.
- Nephritic oedema is mainly due to excessive reabsorption of sodium and water in the renal tubules and not due to protein loss. It is milder as compared to nephrotic oedema.
- In acute tubular injury, which is due to shock or toxic chemicals, tubules lose their capacity for selective renal concentration of the glomerular filtrate resulting in increased reabsorption and oliguria.

Q. Write briefly on cardiac oedema.

Ans. Cardiac oedema is mostly a manifestation of congestive heart failure, and occurs due to activation of a series of mechanisms that increase venous capillary pressure, promote sodium and water retention by the kidneys and expansion of the extracellular fluid (see Flowchart 4.2 for the pathogenesis of oedema).

Q. Write briefly on pulmonary oedema.

Ans. Defined as fluid accumulation in the air spaces and parenchyma of the lungs, pulmonary oedema may lead to respiratory failure due to impaired gas exchange. It is mainly of two types—

1. **Cardiogenic pulmonary oedema** (caused by congestive cardiac failure or left ventricular failure which lead to inadequate removal of blood from the pulmonary circulation)
2. **Noncardiogenic pulmonary oedema** (caused by injury to lung parenchyma or vasculature of the lung)

Q. Write briefly on subcutaneous oedema.

Ans. Subcutaneous oedema can be diffused or localized to the most dependent part of the body positioned at the greatest distance below the heart (legs while standing and the sacrum while recumbent). This type of oedema is called **dependent oedema** and is **pitting** in nature **(ie,** pressure over oedematous subcutaneous tissue displaces the interstitial fluid, leaving a finger-shaped depression).

Q. Write briefly on cerebral oedema.

Ans. **Cerebral oedema** can be localized (eg, due to a space occupying intracranial lesion like an abscess or tumour) or generalized (due to extensive brain pathology or injury). The latter causes narrowing of the sulci while the gyri are swollen and flattened against the skull.

Q. Differentiate between cardiac and renal oedema.

Ans. The differences between cardiac and renal oedema are tabulated in Table 4.2.

TABLE 4.2. Contrasting features of cardiac and renal oedema

Features	Cardiac oedema	Renal oedema
Causes	CHF and right-sided heart failure	Nephritic and nephrotic syndrome/acute tubular injury/necrosis
Mechanism	Decreased cardiac output	Hypoalbuminemia and decreased plasma oncotic pressure
Clinical	• Dependent oedema, the distribution of which changes with posture • Mainly pedal or sacral; later generalized	First observed around face, eyes, ankle and genitalia
Serum albumin	Normal	Decreased
Proteinuria	Absent	Present

Q. Define and compare hyperaemia and congestion. Write briefly on the pathogenesis and outcomes of chronic venous congestion.

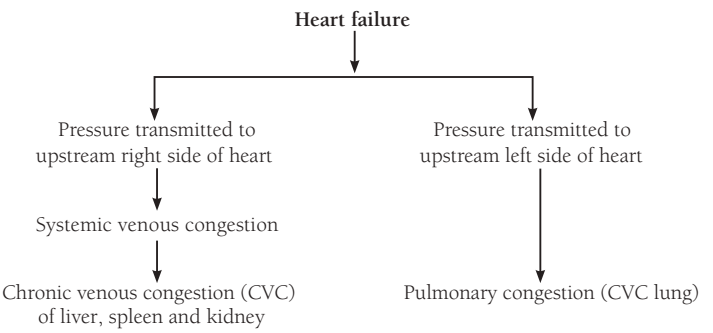
Ans. Hyperaemia is defined as local increase in volume of blood in a particular tissue due to arteriolar dilatation, eg, increased inflow in skeletal muscle during exercise.
Congestion is a passive process resulting from impaired outflow from a particular organ/tissue. It may occur systemically (in cardiac failure) or locally (in an isolated venous obstruction). Congestion and oedema can occur together since capillary blood congestion can lead to increased fluid transudation causing oedema.

The contrasting features of hyperaemia and congestion are summarized in Table 4.3 given below.

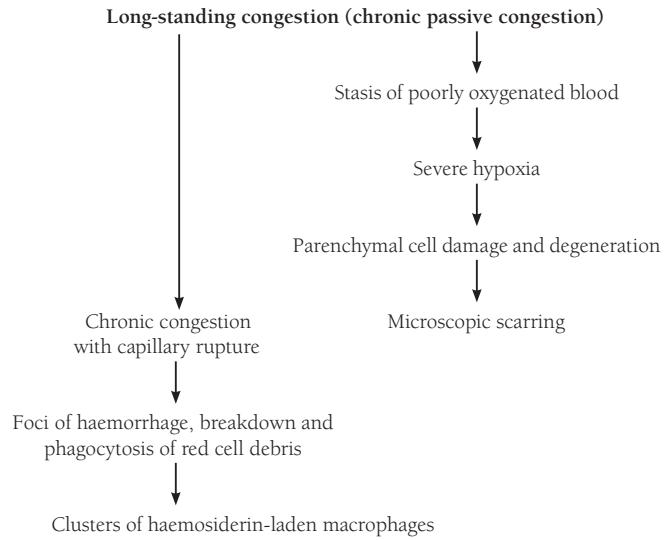
TABLE 4.3. Differences between hyperaemia and congestion

Features	Hyperaemia	Congestion
Definition	Characterized by increased blood flow due to *arteriolar dilatation*	Characterized by blood pooling due to impaired outflow/drainage from tissue
Nature of process	Active	Passive
Appearance	Red	Bluish-red/cyanosed
Type of blood	Oxygenated	Deoxygenated; tissue hypoxia present
Oedema	Absent	Present
Examples	Menopausal flush, muscular exercise, high-grade fever, etc.	Local: portal venous obstruction in cirrhosis of liver; systemic: right-sided heart failure

The pathogenesis and outcomes of chronic venous congestion are depicted in Flowchart 4.3A and B.



FLOWCHART 4.3A. Pathogenesis of chronic venous congestion.



FLOWCHART 4.3B. Outcomes of chronic congestion.

Q. Write briefly on the pathogenesis and clinicopathological features of pulmonary congestion.

Ans. Pulmonary congestion is defined as accumulation of fluid within the pulmonary interstitium as well as alveoli. It may be classified into 'acute or chronic' types based on duration.

Acute Pulmonary Congestion
This may be cardiogenic or noncardiogenic in origin.

Gross morphology
Lungs are enlarged; cut section shows frothy, blood-stained fluid (air in combination with oedema fluid and red cells).

Microscopic Features
The main histopathological features of acute pulmonary congestion are:
- Alveolar septal oedema
- Engorged septal capillaries
- Focal intra-alveolar haemorrhages

Chronic venous congestion (CVC) lung (Fig. 4.2)
Long-standing pulmonary venous congestion occurs due to left-sided heart failure (eg, rheumatic mitral stenosis), which results in increased pulmonary venous pressure.

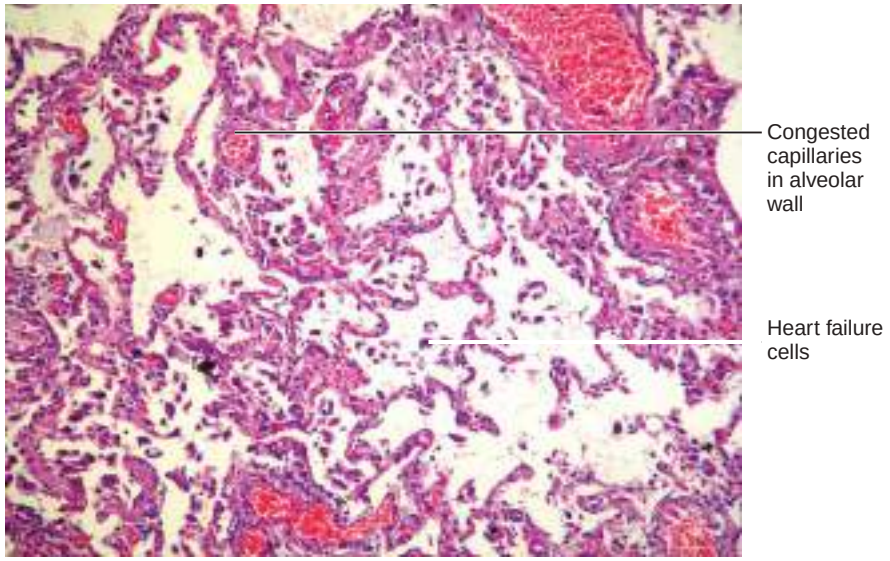


FIGURE 4.2. CVC lung showing congested and widened alveolar septae with hemosiderin-laden macrophages in the alveolar spaces (H&E; 200×).

Gross morphology

- The lungs are heavier and firmer than normal.
- Cut surface is dark brown in colour (**brown induration**) with oozing of frothy, blood-tinged material.

Microscopic features

- Dilatation and congestion of septal capillaries
- Intra-alveolar haemorrhage (occurs due to rupture of congested capillaries)
- RBC breakdown produces hemosiderin which is taken up by alveolar macrophages. These hemosiderin-laden macrophages present in alveolar lumina are called **heart failure cells** (siderophages).
- Thickening and fibrosis of alveolar septae is eventually seen.

Q. Write briefly on the pathogenesis and clinicopathological features of congestive splenomegaly.

Ans. Long-term venous outflow obstruction leads to congestive splenomegaly (splenic enlargement and congestion). Its causes include portal hypertension (may be due to thrombosis of hepatic veins; also called to Budd-Chiari syndrome), cirrhosis, congestive heart failure or stenosis/thrombosis of the portal or splenic veins.

Gross morphology

Enlarged, tense and cyanotic spleen with thickening and fibrosis of capsule

Microscopic features (Fig. 4.3)

- Congestion of red pulp and sinusoids ultimately causing haemorrhage
- Organization of haemorrhage results in formation of siderotic nodules (**Gamna-Gandy bodies**)

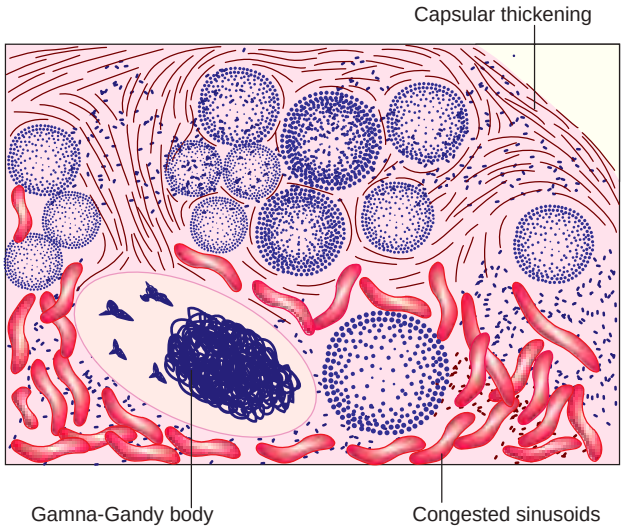


FIGURE 4.3. Section from spleen showing dilated and congested sinusoids with fibrosis and Gamna-Gandy bodies.

Q. Write briefly on the pathogenesis and clinicopathological features of hepatic congestion.

Ans. Congestive hepatopathy refers to hepatic manifestations attributable to right-sided heart failure, Budd-Chiari syndrome, hepatic sinusoidal obstruction syndrome, hepatic infarction and ischaemic hepatitis. Passive congestion often coexists with reduced cardiac output. **Acute hepatic congestion** is characterized by:

- Dilated, distended central vein and sinusoids
- Central hepatocyte degeneration
- Fatty change in periportal hepatocytes (periportal hepatocytes experience less hypoxia because of proximity to hepatic arterioles, and therefore develop fatty change only)

In **chronic passive congestion** of liver (Fig. 4.4), the central region of hepatocytic lobules appears grossly red–brown and slightly depressed with surrounding zones of uncongested liver (***nutmeg appearance***). Central portion of hepatocytes being least

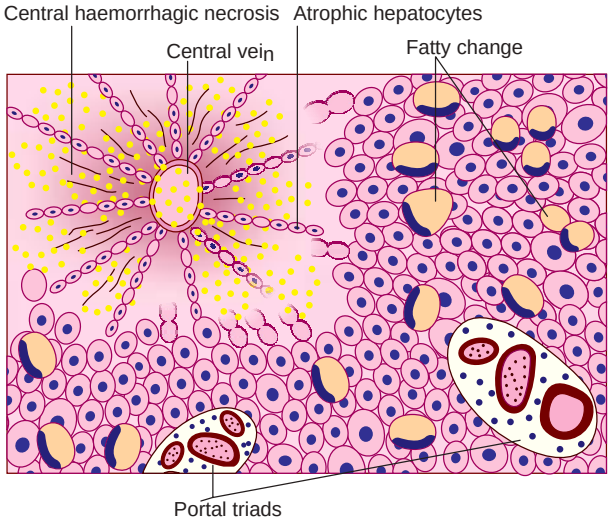


FIGURE 4.4. Photomicrograph of chronic passive congestion of liver showing centrilobular necrosis with loss or drop out of hepatocytes and peripheral fatty change.

perfused, undergoes centrilobular necrosis with loss or drop out of hepatocytes. Severe long-standing congestion may lead to grossly evident hepatic fibrosis (***cardiac cirrhosis***).

Q. Define and describe types of haemorrhage. Write in detail on haemostasis.

Ans. Extravasation of red cells due to vessel rupture is called haemorrhage.

- It may be external (**external haemorrhage**) or enclosed within the tissue (when it is called a **haematoma**).
- Haematoma may be insignificant (**bruise**) or large enough to cause fatality, eg, **a large retroperitoneal haematoma** or an **internal (visceral) haematoma**.
- Minute 1–2 mm haemorrhages into the skin, mucous membranes or serosal surfaces are denoted as **petechiae** (typically seen with locally increased intravascular pressure, low platelet counts and defective platelet function).
- Larger >3 mm haemorrhages are called **purpura** (associated with the same disorders as petechiae and also occur secondary to trauma, vasculitis or increased vascular fragility).
- Still larger >1–2 cm subcutaneous haematomas or bruises are called **ecchymoses** (generally seen after trauma).
- Accumulation of blood in body cavities may be called **haemothorax, haemopericardium, haemoperitoneum** and **haemarthrosis (joints)** depending on the cavity involved.

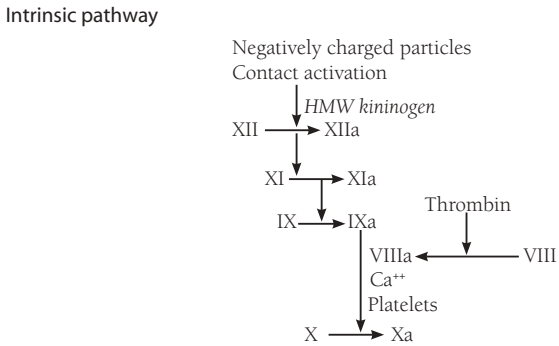
Haemostasis

Haemostasis is the mechanism which maintains the integrity of the circulatory system after vascular damage. Normal haemostatic mechanism of the body has three components:

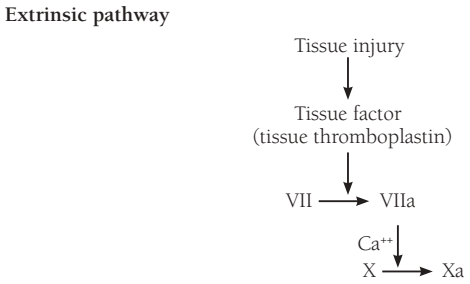
1. **Vascular component:** This involves a reflex spasm of the injured vessel (vasoconstriction), which minimizes the blood loss.
2. **Platelet component:** Platelets are anucleate discoid structures derived from marrow megakaryocytes. The cytoplasm of platelets contains three major types of storage granules:
   (i) Alpha granules containing a variety of proteins like fibrinogen and von Willebrand factor
   (ii) Dense granules containing serotonin, ADP and calcium
   (iii) Lysosomal granules containing acid hydrolases

Following vessel constriction, platelets adhere to the vessel wall and also aggregate to form a platelet plug which seals off the vascular breach and arrests haemorrhage (**primary haemostasis**). This is followed by activation of the coagulation cascade and fibrin deposition (**secondary haemostasis**).

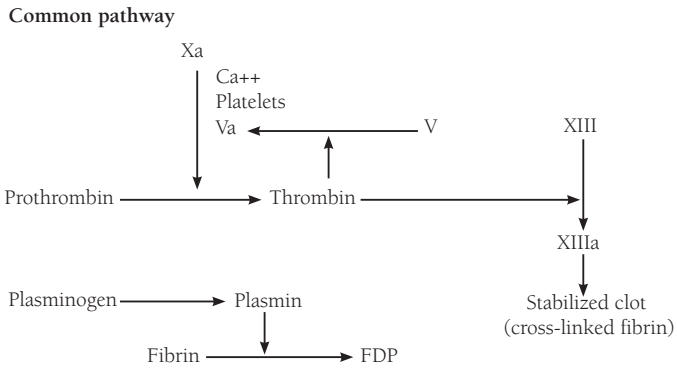
3. **Components of the coagulation cascade:** Coagulation cascade includes three pathways, namely, the **intrinsic** (Flowchart 4.4), **extrinsic** and the **common pathways** (Flowcharts 4.5 and 4.6). Intrinsic pathway is assessed in vitro by the activated partial thromboplastin time (aPTT). Extrinsic pathway is assessed by the prothrombin time (PT). The coagulation factors involved in the different pathways are tabulated in Table 4.4.



FLOWCHART 4.4. Intrinsic pathway of coagulation.



FLOWCHART 4.5. Extrinsic pathways of coagulation.



FLOWCHART 4.6. Common pathways of coagulation.

Inhibitors of Coagulation

The natural inhibitors of coagulation include

- Anti-thrombin III
- α-macroglobulin
- Heparin cofactor II
- Protein C and protein S

Fibrinolytic System

The physiological function of the fibrinolytic system is to digest deposits of fibrin (thrombi).

TABLE 4.4. Coagulation factors

I	Fibrinogen
II	Prothrombin
V	Proaccelerin
VII	Proconvertin
VIII	Anti-haemophilic factor
IX	Christmas factor
X	Stuart-Prower factor
XI	Plasma Thromboplastin antecedent
XII	Hageman factor
XIII	Fibrin stabilizing factor

Prekallikrein: Fletcher factor
HMW kininogen: Fitzgerald factor

Q. Write briefly on the factors contributing to thrombus formation.

Ans. Thrombosis is the process of formation of a solid mass in the circulation constituted by platelets, fibrin and other entrapped blood elements. Vessel wall injury induces rapid recruitment of the circulating platelets to the site of injury, where they initiate formation of a thrombus. There are three major contributors to thrombus formation:

1. **Endothelial injury, which may be secondary to:**
   (a) Myocardial infarction
   (b) Ulcerated atherosclerotic plaques
   (c) Cardiac surgery
   (d) Myocarditis

(e) Infected valve disease
(f) Prosthetic valves
(g) Radiation injury
(h) Chemical agents (smoking, hypercholesterolaemia, homocysteineamia, bacterial toxins and endotoxins)

2. **Alteration in normal blood flow (stasis or turbulence):**
Stasis is typically seen in hyperviscosity syndromes and polycythemia; whereas, **turbulence** is commonly associated with hypertension. Alterations in normal flow result in:
(a) Disruption of laminar flow
(b) Decreased hepatic clearance of activated coagulation factors
(c) Damage to endothelium
3. **Conditions predisposing to hypercoagulability:**
(a) Genetic:
(i) Deficiency of antithrombotic factors like AT III, proteins C and S, Methylene tetrahydrofolate reductase (MTHFR) gene mutation and defects in fibrinolysis.
(ii) Increased prothrombotic factors as in Factor V mutation/factor V Leiden (activated protein C resistance); prothrombin G20210A mutation (excessive levels of prothrombin); high levels of factors VII, XI, IX, VIII, von Willebrand factor and fibrinogen and homocysteinuria.
(b) Acquired:
(i) **Venous stasis:** Prolonged immobilization and congestive cardiac failure
(ii) **Increased platelet activation:** Cancers, acute leukaemias, myeloproliferative disorders, paroxysmal nocturnal haemoglobinuria, prosthetic cardiac valves, atrial fibrillation, myocardial infarction and thrombotic thrombocytopenic purpura
(iv) **Increased hepatic synthesis of coagulation factors or reduced anticoagulant synthesis:** Oral contraceptives, pregnancy, etc.
(v) **Antiphospholipid syndrome**
(vi) **Tissue injury:** Surgery, fracture and extensive burns

Q. Write briefly on the morphology of a thrombus.

Ans. Thrombi are grey-white, friable, tangled strands of fibrin and platelets, which may form anywhere in the cardiovascular system, as in cardiac chambers, arteries and veins and capillaries.

General Features of Thrombi

- Different sizes and shapes of thrombi may be seen, dictated by:
  - Site of origin
  - Circumstances leading to their development
- **Cardiac thrombi** mostly develop in the regions of turbulence and at sites of endocardial injury (atrial appendages, endocardial surface of a myocardial infarct and cardiac valves).
- **Thrombi in cardiac chambers** or **aorta** show the presence of laminations or **lines of Zahn** (paler layers of fibrin and platelets alternating with darker layers of RBCs).
- **Thrombi in smaller arteries** or **veins** do not show lines of Zahn.
- **Mural thrombi** are attached to one wall of an underlying structure, usually capacious lumina of heart chambers and aorta.
- **Arterial thrombi** are usually occlusive when they involve smaller vessels; large vessels, eg, iliac and common carotid tend to have mural thrombi.
- **Venous thrombi (phlebothrombosis)** are invariably occlusive and contain a large RBC component, because these are formed in a relatively static environment. These are also called **red** or **stasis thrombi**. Other features of venous thrombi are as follows:
  - Lines of Zahn are not well developed.
  - Mostly affect veins of lower extremity (90% cases).
  - May be confused with post-mortem clots.
  - Always have a point of attachment to the underlying structure, firmest at the point of origin.
- Contraction of a thrombus gives way to a slit-like lumen which restores blood flow leading to propagation of the thrombus upstream and downstream.

Q. Differentiate between arterial and venous thrombi.

Ans. Differences between arterial and venous thrombi are tabulated in Table 4.5.

TABLE 4.5. Differences between arterial and venous thrombi

Features	Arterial thrombi	Venous thrombi
Sites	Common in coronary, cerebral, iliac and femoral arteries (vessels with active blood flow)	Superficial varicose veins and deep veins of leg, eg, femoral and iliac veins (vessels with less active blood flow)
Pathogenesis	Due to endothelial injury (as in atherosclerosis or turbulent blood flow)	Due to venous stasis
Progression	Grows in a retrograde direction from point of attachment	Extends in the direction of blood flow
Occlusion	Do not occlude lumen completely	Invariably occlusive
Gross	Grey-white, friable, prominent lines of Zahn	Dark red with fibrin strands; lines of Zahn less prominent or absent
Microscopy	Lines of Zahn show paler layers of fibrin and platelets alternating with darker layers of RBCs	Constituted by more of RBCs and less of fibrin
Complications	Ischaemia/infarction of vital organs	Embolism, oedema, ulceration

Q. Differentiate between antemortem and post-mortem clot.

Ans. Differences between antemortem and post-mortem clot are tabulated in Table 4.6.

TABLE 4.6. Differences between antemortem and post-mortem clot

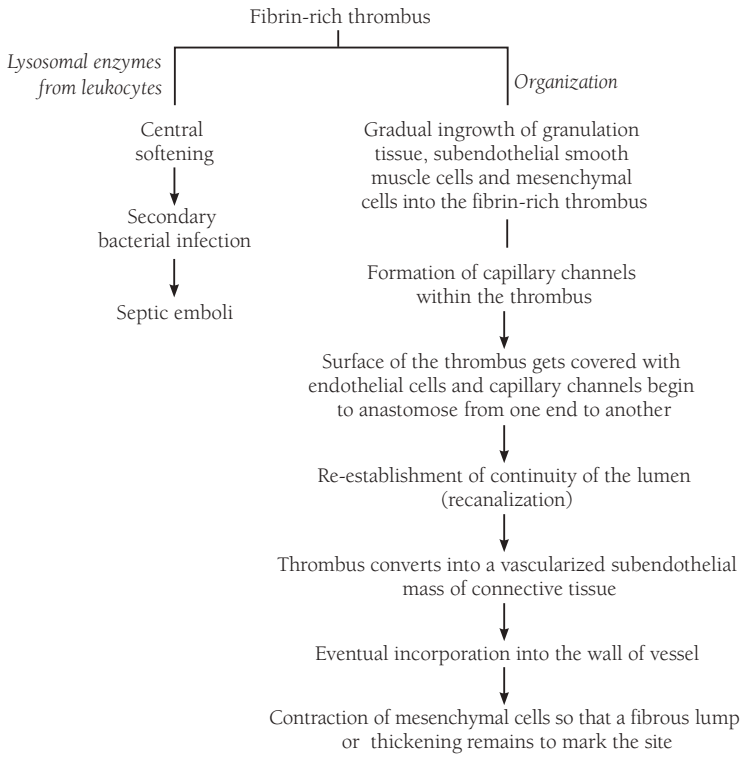
Features	Antemortem clots/thrombi	Post-mortem clots
Origin	Formed as part of normal haemostasis or pathological derangement of clotting pathway in a living person	Form in a dead person due to sedimentation and settling down of blood components
Gross	• Dry, granular, firm and friable • **Lines of Zahn** are prominent in arterial thrombi	• Gelatinous, soft and rubbery • Dark red, dependent portion of the clot is called **currant jelly** and yellow supernatant, free of red cells is called **chicken fat**
Shape	Do not form a cast of the vessel	Take the shape of the vessel or its bifurcation forms a cast of the vessel
Attachment to vessel wall	Present; strong	Very weak
Location	Anywhere in the body	In dependent parts of the body

Q. Write briefly on fate of a thrombus.

Ans. A thrombus may undergo:

1. Propagation (accumulation of additional platelets and fibrin leading to progression)
2. Embolization (propagating tail fragments give rise to emboli)
3. Dissolution (fibrinolysis usually on the first or second day)
4. Organization and recanalization (ingrowth of endothelial cells, smooth muscle and fibroblasts)
5. Inflammation and fibrosis (central liquefaction, bacterial seeding and influx of inflammatory cells)

Sequence of events in evolution of a thrombus (Flowchart 4.7):



FLOWCHART 4.7. Sequence of events in evolution of a thrombus.

Q. Classify emboli.

Ans. Emboli are classified based on:

(a) Physical state of emboli
- Solid: Atheromatous, thromboemboli and tumour emboli
- Liquid: Fat, bone marrow and amniotic fluid emboli
- Gaseous: Air emboli (seen in decompression sickness)

(b) Site of origin
- Cardiac emboli (left side of heart)
- Arterial emboli (atheromas and aneurysms)
- Venous emboli (deep vein thrombosis)
- Lymphatic emboli (tumour emboli)

(c) Presence or absence of secondary infection
- Sterile/bland emboli
- Septic emboli

(d) Flow
- **Paradoxical emboli/crossed emboli:** Emboli crossing over from venous circulation to arterial circulation or vice-versa; deep leg vein emboli cross to pulmonary circulation and then to systemic arterial circulation.
- **Retrograde emboli:** Travel against the direction of blood flow, eg, retrograde spread of prostatic carcinoma to spine via intraspinal veins. Increased pressure in the body cavities during coughing or straining carries emboli from large thoracic ducts and abdominal veins.

Q. Write briefly on the aetiopathogenesis and complications of pulmonary embolism.

Ans. It is one of the most common problems encountered in hospitalized or bedridden patients. It may be fatal, causing occlusion of pulmonary artery and its branches.

Sources

Thrombi from large veins of lower legs (most commonly deep vein thrombosis and less commonly superficial veins of leg and pelvis).

Pathogenesis

- Thrombus as a whole, or its loosely attached tail, gets detached from its origin, and is carried through venous channels to the right side of heart where it enters pulmonary circulation.
- If large enough, it gets impacted at the bifurcation of the main pulmonary artery (**saddle embolus**) or lodges in the right ventricle or its outflow tract.
- Multiple, small emboli may occlude smaller pulmonary vessels.
- Emboli may pass through atrial or ventricular septal defects from right side to left side of the heart to enter into arterial circulation (**paradoxical emboli**).

Clinical Features

Cough, severe pleuritic pain, shortness of breath, occasionally haemoptysis and haemorrhagic pleural effusion

Sequelae and Complications

Most emboli remain *silent* and undergo resolution. Others may cause

- Sudden death due to right-sided heart failure (*cor pulmonale*) or cardiovascular collapse (obstruction of >60% of the pulmonary circulation or massive pulmonary embolism)
- *Pulmonary haemorrhage* due to obstruction of terminal branches of pulmonary artery
- *Pulmonary infarction* due to obstruction of end-arteriolar pulmonary branches
- *Pulmonary hypertension* with right-sided heart failure (multiple emboli clogging pulmonary capillary circulation)

Q. Write briefly on fat embolism.

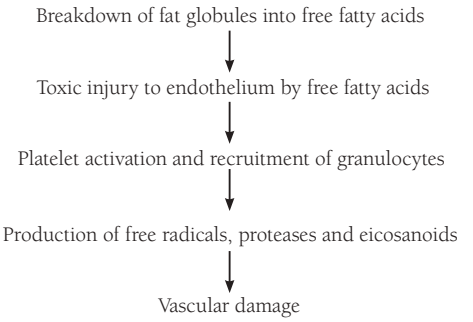
Ans. Fat embolism is defined as obstruction of arterioles and capillaries by fat globules with or without marrow elements.

Causes

- Trauma to long bones or soft tissue (fracture with embolization of fatty marrow)
- Extensive burns
- Pancreatitis
- Diabetes mellitus
- Vigorous cardiopulmonary resuscitation

Pathogenesis

- *Mechanical obstruction*: Fat globules occlude pulmonary or systemic circulation (brain, kidneys and other organs) to cause platelet and RBC aggregation leading to hypoxia of that tissue/organ.
- *Biochemical injury* (Flowchart 4.8)



FLOWCHART 4.8. Biochemical basis of cellular injury in fat embolism.

Clinical Features

Clinical manifestations appear within 1–3 days of trauma and include

- Tachypnoea, dyspnoea and tachycardia (**pulmonary insufficiency**)
- Irritability, restlessness, delirium and coma (**neurological effects**)
- Diffuse petechial rash in nondependent areas (due to thrombocytopaenia resulting from **platelet consumption**)
- Anaemia (**due to aggregation of RBCs** and **microangiopathic haemolysis**)

Q. Write briefly on systemic thromboembolism.

Ans. Majority of systemic emboli originate from intracardiac mural thrombi (two-thirds from left ventricular wall infarcts; one-fourth from left atrial dilatation and fibrillation and the remaining from aortic aneurysms, atheromas, valvular vegetations and paradoxical emboli). About 10–15% are of ambiguous origin. Venous emboli mostly travel to the lungs; arterial emboli travel to different sites, most commonly lower extremities or brain.

Q. Write briefly on amniotic fluid embolism.

Ans. Amniotic fluid embolism is a cause of maternal morbidity during labour and immediate postpartum period, and has a mortality of up to 20–40%.

Pathogenesis

Caused by infusion of amniotic fluid with all its contents (fetal cells and debris) into maternal circulation due to tears in placental membrane or rupture of uterine vessels.

Clinical Findings

- Sudden respiratory distress
- Deep cyanosis
- Hypotensive shock
- Seizures, convulsions, coma and death

Microscopic Features

- Pulmonary microcirculation shows fetal skin, squamous cells, lanugo hair and fat from vernix caseosa, mucin from fetal respiratory tract or GIT.
- There is pulmonary oedema and diffuse alveolar damage and haemorrhage.

Causes of Death

- Mechanical blockage of pulmonary circulation
- DIC

- Anaphylactic reaction to amniotic fluid
- Haemorrhage (if the patient survives the initial phase, she may bleed extensively due to disseminated intravascular coagulation or DIC)

Q. Write briefly on air embolism.

Ans. Air bubbles in the circulation can obstruct vascular flow and cause ischaemic injury.

Causes

Air can be sucked into the arterial and venous circulation in the following conditions:

- Operative procedures (surgery in the head and neck and cardiothoracic regions or obstetric manipulations)
- Trauma, particularly penetrating chest wall injury
- Decompression sickness
- Intravenous infusions
- Angiography/arteriography

Arterial air embolism can also be associated with a paradoxical embolus that can travel to the arterial circulation from the venous side across a patent foramen ovale or arteriovenous shunts.

Caisson disease is a specialized form of air embolism, which occurs when a person decompresses suddenly across areas with major pressure differences.

Pathogenesis

- Decompression sickness or caisson disease occurs in individuals exposed to sudden changes in atmospheric pressure, eg, scuba and deep-sea divers.
- When air is breathed at high pressure, large amounts of gas particularly nitrogen, dissolves in blood and tissue.
- If the diver rapidly ascents from water (depressurizes), the nitrogen bubbles out of the blood to form gas emboli. These bubbles lodge in the blood vessels of muscles and joints causing 'bends' and oedema and haemorrhage in lungs causing respiratory distress or 'chokes'.
- Chronic decompression sickness is seen in workers of pressurized vessels used in bridge construction and may cause multiple foci of avascular necrosis in femur, tibia and humerus.

Q. Define and classify infarcts.

Ans. An infarct is an area of ischaemic coagulative necrosis caused by occlusion of arterial supply or venous drainage.

- Almost all infarcts result from thromboembolic events affecting arterial circulation.
- Others may result from vasospasm, twisting of vessels (testicular torsion or bowel volvulus), vascular compression (by oedema, entrapment in a hernial sac or tumour), and traumatic vessel rupture.
- Venous thrombosis usually results in venous congestion; can, however, cause infarction in rare instances (as in organs with a single venous outflow channel, eg, testes and ovaries).

Infarcts are classified on the basis of the following:

1. **Colour (amount of haemorrhage)**
   (a) Red or haemorrhagic infarcts
   (b) White or anaemic infarcts
2. **Presence or absence of microbial infection**
   (a) Septic infarcts
   (b) Bland infarcts

Q. Differentiate between red and white infarcts.

Ans. Differences between red/haemorrhagic and white/pale/anaemic infarcts are tabulated in Table 4.7.

TABLE 4.7. Differences between red/haemorrhagic and white/pale/anaemic infarcts

Features	Red infarct	White infarct
Organs involved	Spongy organs like lung and gastrointestinal tract	Solid organs, like heart, spleen and kidney
Cause	*Venous occlusion* Seen • In loose tissues that allow collection of blood or tissues with dual blood supply (lungs, GIT). Haemorrhage seeps into such an infarct when flow is re-established. • In tissues that were previously congested due to sluggish venous outflow • When blood flow is re-established in a site with previous arterial occlusion, eg, after coronary angioplasty	*Arterial obstruction* Seen in solid organs where the solidity of the tissue prevents haemorrhage that can seep through from adjoining capillaries and tissues with end arterial circulation
Morphology	Congested and red due to haemorrhage; turns brown and firm with time but never appears pale. Hemosiderin-laden macrophages are present in large numbers.	Becomes progressively pale
Margins	Not sharply defined	Sharply defined
Oedema	Present	Absent

Q. Write briefly on renal infarcts.

Ans. Renal infarcts are often multiple and pale in appearance.

- May be bilateral.
- Have a wedge-shaped base resting under the capsule with the apex towards medulla.
- A narrow rim of renal tissue under the capsule is spared because its blood supply is derived from capsular vessels.
- Microscopically, affected area shows coagulative necrosis due to hypoxia.

Q. Define and classify shock. Describe its pathogenesis and clinical presentation.

Ans. Shock is defined as a clinical state of cardiovascular collapse characterized by the inadequate perfusion of the cells and tissues resulting in hypotension and cellular hypoxia. If uncompensated, it may lead to impaired cellular metabolism and death.

Aetiology and Classification

1. **Hypovolaemic** Characterized by reduction in the circulating blood volume. Causes include
   (a) Severe haemorrhage (trauma and surgery)
   (b) Fluid loss (severe burns, crush injuries, vomiting and severe diarrhoea)
2. **Cardiogenic shock:** Due to failure of the myocardial pump. Results from:
   (a) Deficient emptying
      (i) Myocardial infarction
      (ii) Rupture of the heart
      (iii) Cardiac arrhythmias

(b) Deficient filling
Cardiac tamponade
(c) Obstruction to outflow
(i) Pulmonary embolism
(ii) Ball valve thrombus

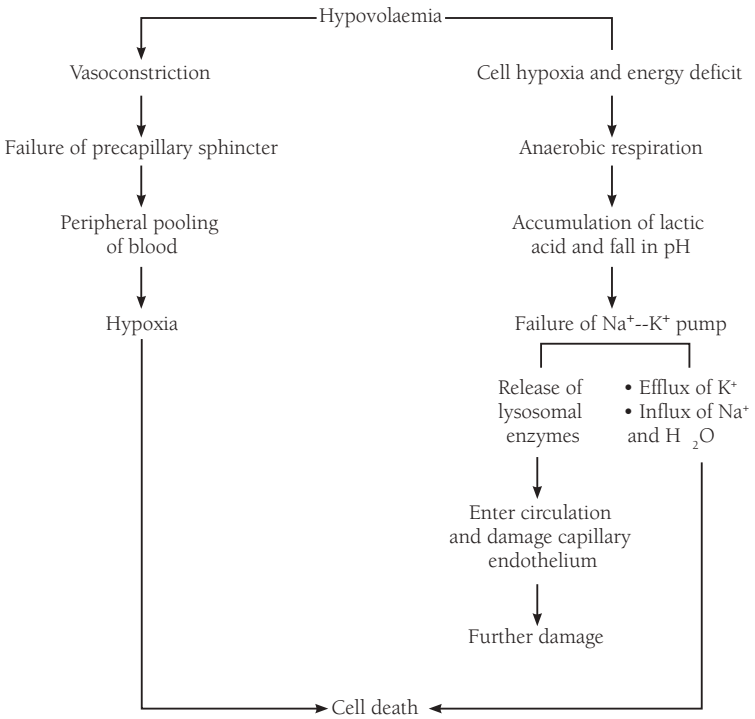
3. **Neurogenic shock:** Occurs due to loss of vascular tone and peripheral pooling of blood following anaesthesia or spinal cord injury.
4. **Anaphylactic shock:** Occurs when an allergic response triggers a quick release of mast cell mediators in large quantities (histamine, prostaglandins and leukotrienes) leading to systemic vasodilatation (associated with hypotension), increased vascular permeability and bronchoconstriction (leading to difficulty in breathing). Shock can lead to death in a matter of minutes if left untreated.
5. **Septic shock:** Occurs when there is widespread endothelial injury and activation due to
(a) Severe bacterial infections
(i) Predominantly Gram-positive infections (*streptococci* and *pneumococci*)
(ii) Gram-negative infections (*E. coli, Proteus, Klebsiella* and *Pseudomonas*)
(b) Fungal or rickettsial sepsis
(c) Super antigens (polyclonal T lymphocyte activators that induce release of high levels of cytokines that lead to vasodilatation, hypotension and shock)

Pathogenesis of Hypovolaemic Shock (Flowchart 4.9)

Pathogenesis of Cardiogenic Shock

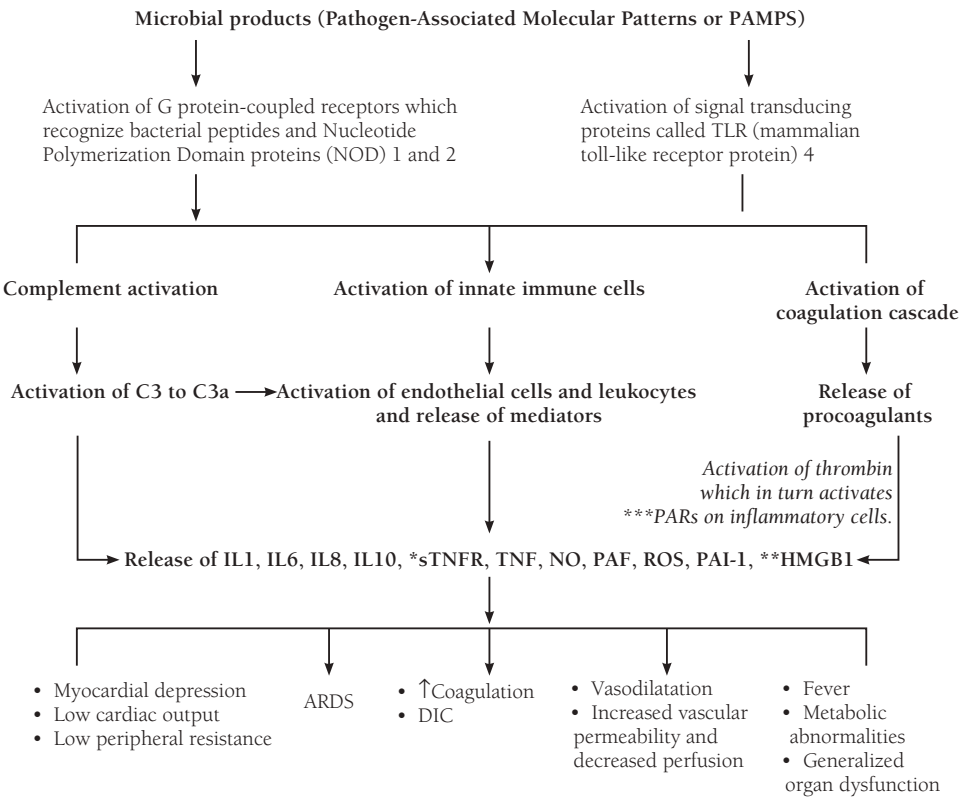
Cardiogenic shock entails:

- Acute circulatory failure with sudden fall in cardiac output causing reduced effective circulating blood volume
- Reduced supply of oxygen to the cells and tissue with resultant anoxia



FLOWCHART 4.9. Pathogenesis of hypovolaemic shock.

Pathogenesis of Septic Shock (Flowchart 4.10)



***sTNFR—Soluble TNF receptor**
****HMGB1—High mobility group box 1 protein**
*****PARs—Protease-activated receptors**

FLOWCHART 4.10. Pathogenesis of septic shock.

Stages of Shock

1. **Nonprogressive (initial, compensated and reversible) shock:**
   (a) Attempt is made to maintain adequate cerebral and coronary blood supply by redistribution of blood so that vital organs (brain and heart) are perfused and oxygenated.
   (b) Activation of neurohumoral mechanisms leads to widespread vasoconstriction and fluid conservation by the kidney. Neurohumoral mechanisms involved include
      (i) Activation of baroreceptors and chemoreceptors
      (ii) Activation of renin–angiotensin–aldosterone system
      (iii) ADH release
      (iv) Release of catecholamines
      (v) Vascular autoregulation—in response to hypoxia and acidosis, regional blood flow to the heart and brain preserved by vasodilatation of coronary and cerebral circulation
2. **Progressive decompensated shock:** If the underlying cause is not corrected or the patient has pre-existing cardiovascular disease, persistence of shock leads to
   (a) Pulmonary hypoperfusion and tachypnoea
   (b) Tissue anoxia initiating anaerobic glycolysis leading to lactic acidosis and ineffective vasomotor response causing peripheral pooling and vasodilatation
3. **Decompensated (irreversible) shock:** *Widespread cell injury leads to*
   (a) Progressive decrease in blood pressure due to decrease in cardiac output
   (b) Metabolic acidosis

(c) Adult respiratory distress syndrome (ARDS)
(d) Ischaemic cell death of brain, heart and kidney

Causes of Irreversibility

- Widespread vasoconstriction starts as a compensatory mechanism but its persistence causes anoxia of tissue.
- Anoxic damage to the endothelial lining causes increased vascular permeability.
- Myocardial ischaemia induces release of myocardial depressant factor (MDF), which causes decreased coronary blood flow.
- Cerebral ischaemia causes depression of vasomotor centre.
- Normally, vasodepressor material (VDM) produced by spleen and skeletal muscle is inactivated in liver. In severe hypoxia of liver, no inactivation of VDM takes place inducing leading to peripheral vasodilatation.
- Release of TNF
- Hypercoagulability of blood

Morphologic Changes in Shock

- Morphologic changes in shock are due to hypoxia resulting in degeneration and necrosis in various organs.
- Major organs affected are brain, heart, lungs and kidneys. Adrenals, GIT and liver are also affected.

1. **Hypoxic encephalopathy**
   - Neurons more prone to hypoxic damage.
   - Cytoplasm of affected neurons becomes intensely eosinophilic and the nucleus pyknotic.
   - Dead and dying nerve cells are replaced by gliosis.
2. **Heart**
   - Heart is more vulnerable to the effects of hypoxia than any other organ.
   - Subepicardial and subendocardial regions show haemorrhage and necrosis.
   - Zonal lesions (contraction bands in the myocytes near the intercalated disc) and distortion of myofilaments may be seen.
3. **Lung**
   Because of dual blood supply, lungs are generally not affected by hypovolaemic shock. In septic shock, lungs may manifest with ARDS (shock lung) or show the following features:
   - Diffuse alveolar damage
   - Interstitial lymphocytic infiltrate and oedema
   - Formation of alveolar hyaline membrane, and thickening and fibrosis of alveolar septae
   - Presence of microthrombi in pulmonary microvasculature
4. **Kidney**
   - Shock may lead to irreversible renal injury resulting in anuria and death.
   - Kidney is swollen and may show acute tubular necrosis and brown tubular casts. The latter are seen in cases of extensive muscle damage due to intravascular haemolysis.
5. **Adrenals**
   - Stress response in shock releases aldosterone, glucocorticoids and catecholamines.
   - Active adrenal cells utilize stored lipids for the synthesis of steroids.
   - Adrenal haemorrhages may be seen in severe shock.
6. **Haemorrhagic gastroenteropathy**
   - Multifocal superficial ulcers are seen.
   - Adjoining bowel mucosa is oedematous and haemorrhagic.
   - Microscopy reveals mucosal and mural infarction.
7. **Liver**
   - Liver is usually enlarged and shows a mottled cut surface.
   - Sections show focal (centrilobular) necrosis and fatty change.

8. **Other organs**
   - Necrotic foci may be seen in lymph node, spleen and pancreas.
   - Patients may succumb to septicaemia due to altered immune status.

Clinical Features of Shock

- Hypotension
- Cold clammy skin
- Rapid, thready pulse
- Shallow and sighing respiration
- Pale face, sunken eyes and weakness
- Uncontrolled sepsis—warm skin due to vasodilatation
- Urinary output less than 30 ml/hour

5

Diseases of Immunity

The **human immune system** is a complex network of signals, which controls responses to antigenic stimulation and protects us from diseases. The components of the immune system are:

- **Antigen-specific** (recognize and act against particular antigens)
- **Systemic** (elicit a response which affects the entire body and is not confined to the initial affected site)
- **Have memory** (recognize and mount an even stronger attack to the same antigen the next time)

The functions of the immune system are:

- To provide resistance against invading pathogens (viruses, bacteria, parasites, etc.) and foreign material (eg, transplanted organ)
- To remove 'worn-out' cells (eg, aged cells or tissue debris from site of injury or disease)
- To provide primary defence against cancer

Inappropriate immune responses may manifest as

- Allergies
- Autoimmune diseases

An **antigen** is a substance (usually a protein) that evokes the production of **antibodies**. An **epitope**, also known as '**antigenic determinant**', is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells or T cells.

An **antibody** is a Y-shaped protein (Fig. 5.1) on the surface of B cells that is secreted into the blood or lymph in response to an antigenic stimulus, such as a pathogen, or a transplanted organ. It binds to a specific antigen and neutralizes it. Antibodies are basically **glycoproteins** which belong to the **immunoglobulin superfamily**. Antibodies (immunoglobulins) have two basic structural units—each with **two large heavy chains** and **two small light chains**. The amino acid sequence in the tips of the 'Y' varies greatly among different antibodies

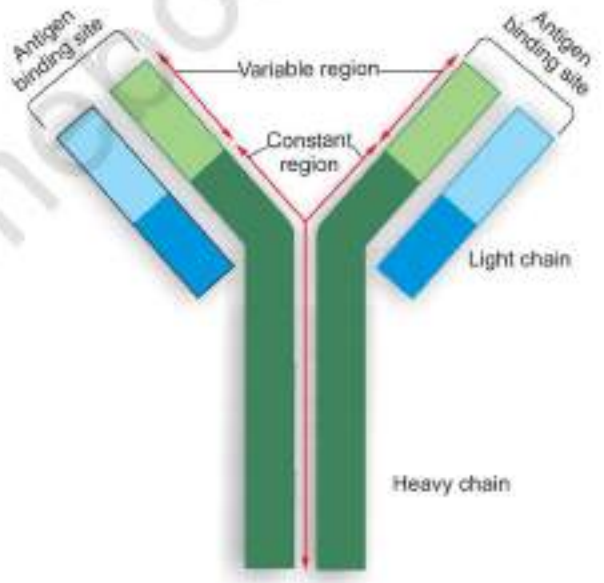
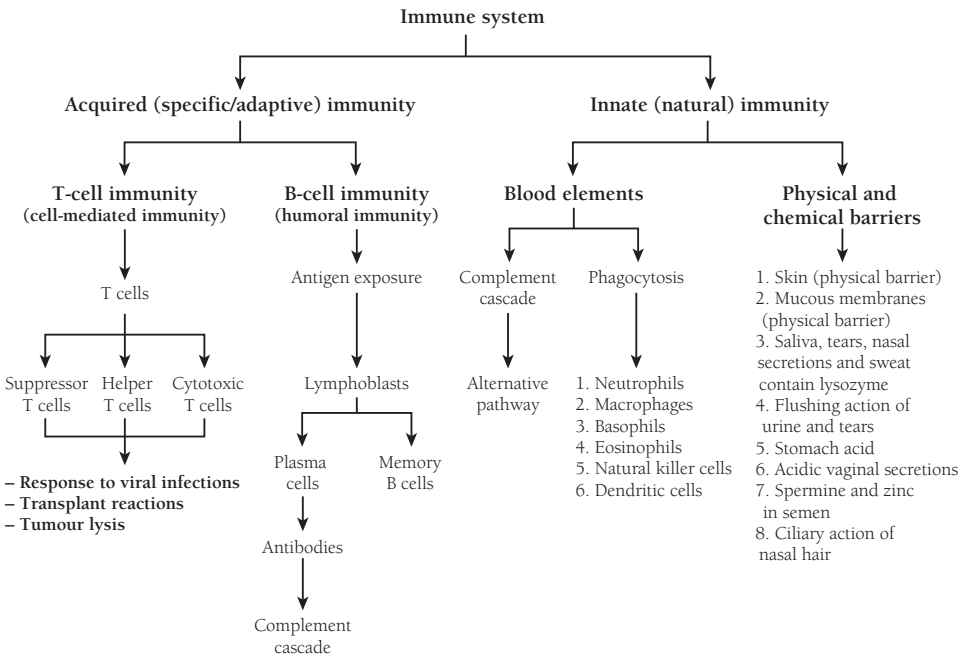


FIGURE 5.1. Structure of an immunoglobulin molecule.

and is labelled the **'variable region'.** It is composed of 110–130 amino acids which give the antibody its specificity for binding antigen. The variable region includes the ends of the light and heavy chains. Digestion with the protease papain cleaves antibody molecules into three fragments. Two fragments are identical and contain the antigen-binding activity. These are termed the **'Fab fragments' (for fragment antigen binding)**. The other fragment contains no antigen-binding activity but was originally observed to crystallize readily, and for this reason was named the **'Fc fragment' (for Fragment crystallizable).** The **constant region** determines the mechanism used to destroy the antigen.

Antibodies are divided into five major classes: IgM, IgG, IgA, IgD and IgE, based on their constant region structure. IgMs have mu-chains; IgAs have alpha-chains; IgEs have epsilon-chains and IgDs have delta-chains. Differences in heavy chain polypeptides determine the function of different immunoglobulins. The polypeptide protein sequences responsible for these differences are found primarily in the Fc fragment. While there are five different types of heavy chains, there are only two main types of light chains: kappa (κ) and lambda (λ). Each antibody binds to a specific antigen. The antigenic substance may be from the external environment or from within the body.

The immune responses of the body are classified into two types (Flowchart 5.1).



FLOWCHART 5.1. Types of immune responses.

1. **Natural or innate**
   - This is the **initial, nonspecific** immune response of the body. Despite the **generalized nature of the response**, it is considered a critical component of the immune system, as defects in it often result in major consequences.
   - The main components of the innate immune system are (1) physical epithelial barriers like skin and mucosal surfaces, (2) granulocytes and macrophages, (3) dendritic cells, (4) a special class of lymphocytes called a natural killer (NK) cells, (5) circulating plasma proteins and (6) chemical barriers present in different bodily secretions (saliva, tears, nasal secretions and sweat contain lysozyme, an enzyme that destroys Gram-positive bacterial cell walls causing cell lysis; vaginal secretions are acidic; spermine and zinc in semen destroy some pathogens and lactoperoxidase is a powerful enzyme found in mother's milk).
   - Pathogens are also prevented from entering the respiratory tract by ciliary action of the tiny nostril hair and the coughing and sneezing reflexes. The flushing actions of tears, saliva and urine also force out pathogens, as does the sloughing off of skin.

- The complement system has several important functions in innate immunity and consists of at least 20 serum glycoproteins, which are activated in a cascade sequence, meaning that activation of a single molecule will lead to thousands of molecules being generated and therefore amplification of the response.

2. **Acquired or adaptive**
   - The **adaptive immune system** is the second line of defence against pathogens that are able to evade or overcome innate immune defences. This is an **antigen-specific** immune response.
   - There are two types of adaptive immune responses: **humoral immunity**, mediated by antibodies produced by B lymphocytes and **cell-mediated immunity** (CMI), mediated by T lymphocytes.
   - The T and B cells of the adaptive immune response are responsible for **long-term memory**. Upon secondary exposure to a specific antigen, the cells of the adaptive immune response exert their effects in a stronger and quicker way than the natural or innate response.

The immune system has several functions, most important of which is self-recognition and non-self-recognition. When the process of self-recognition breaks down and the immune system begins to attack self-antigens, the condition is labelled as **autoimmunity. Examples of autoimmune diseases** include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and diabetes mellitus (DM).

Q. Write briefly on the cells of immune system.

Ans. Cells of the immune system include the following:

Lymphocytes

Lymphocytes express specific receptors for antigens. Their maturation takes place before exposure to this antigen. This is referred to as 'clonal selection'. Lymphocytes of the same specificity are said to belong to the same clone and express the same antigen receptors.

T Lymphocytes

- Thymus-derived cells, which are mediators of CMI.
- Mature T cells constitute 60–70% of circulating lymphocytes and are also present in the paracortical region of lymph node and periarteriolar sheath of the spleen.
- Each T cell is genetically programmed to recognize a specific cell-bound antigen by means of an antigen-specific T cell receptor (TCR).
- In 95% T cells, 'TCR' consists of a disulphide linkage made up of α and β polypeptide chains. In 5% T cells, the disulphide linkage is made up of γ and δ polypeptide chains. The $\alpha\beta$ TCR recognizes peptide antigens presented by major histocompatibility antigens. The $\gamma\delta$ TCR recognizes peptides, lipids and small molecules without the need for antigen presentation by major histocompatibility antigens. $\gamma\delta$ cells are present in the epithelial surfaces (skin, mucosa, GIT) and mainly protect from microbes entering through epithelial surfaces.
- A subset of T cells expresses markers that are also found on NK cells (NK-T cells). The NK-T cells recognize glycolipids displayed by major histocompatibility complex (MHC) like molecule CD1 and their function is inadequately defined.
- 'TCR diversity' is generated by somatic rearrangement of genes coding for α, β and γ, δ chains.
- The enzyme in developing lymphocytes that mediates rearrangement of TCR is the product of RAG1 and RAG2 (recombination activating genes). Inherited defects in RAG proteins results in failure to generate mature lymphocytes.
- Each TCR is noncovalently linked to five polypeptide chains, which form the CD3 complex and ζ-chain dimer (TCR complex). CD3 complex and ζ-chain dimer are identical in all T cells.
- In addition to CD3 proteins, T cells express a variety of other molecules, ie, CD4, CD8, CD2, CD11a, CD28, CD40 and integrins.
- CD4 is expressed in 60% of mature CD3+ T cells and CD8 is expressed in 30% of mature CD3+ T cells.

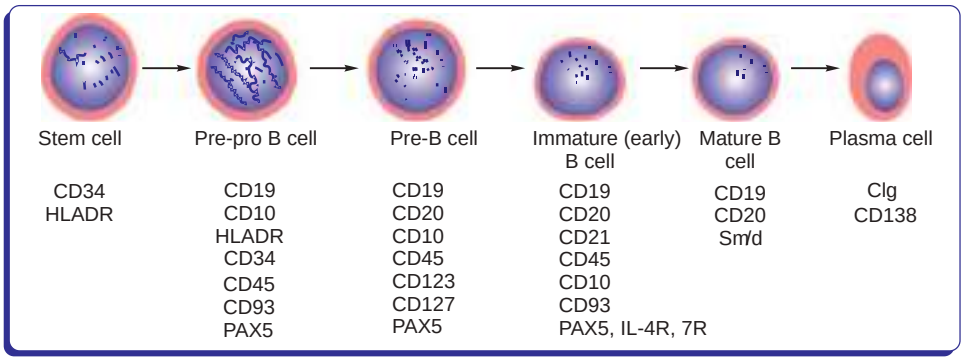


FIGURE 5.2. Ontogeny of B lymphocyte.

B Lymphocytes

- Mediators of humoral immunity which make antibodies against soluble antigens.
- Derived from progenitor B cells produced in the bone marrow (named B cells because they were found to be derived from a lymphoid organ called bursa of Fabricius in chickens; Fig. 5.2).
- Constitute 10–20% of circulating lymphocytes. Also present in lymph nodes (superficial cortex), spleen (white pulp), tonsils, bone narrow and mucosa-associated lymphoid tissue (gastrointestinal tract).
- Naïve B cells recognize antigens and in the presence of helper T cells differentiate into two types: plasma B cells (synthesize immunoglobulins) and memory B cells (remain in secondary lymphoid organs as memory cells; already activated by antigen; they produce quicker responses on later exposure to the same antigen).
- B cells recognize antigen via BCR complex. Each BCR has a unique antigen specificity derived from an RAG-mediated rearrangement of Ig genes. Analysis of Ig gene rearrangement is useful in the identification of monoclonal B cell tumours. Components of BCR complex include:
  - Membrane-bound surface antibodies (IgM and IgD which are the antigen-binding components)
  - Igα and Igβ (required for signal transduction)
  - Complement receptor CD21 (EBV receptor)
  - Fc receptor
  - CD40 (member of TNF family)

Dendritic Cells

- Dendritic cells are immune cells whose main function is to process antigen material and present it to other cells of the immune system. They initiate T cell responses against protein antigens; express high levels of MHC molecules and possess receptors for pathogens (like TLRs and lectins).
- They grow branched projections, the dendrites, which give the cell its name.
- *Types*:
  - *Interdigitating dendritic cells*: Nonphagocytic cells that express high levels of MHC class II and T cell costimulatory molecules. They are present mainly in the epidermis (where a specialized immature dendritic cell type is called Langerhans cell) and the inner lining of the nose, lung, stomach and intestine. Once activated, they migrate to the lymphoid tissues where they interact with T and B cells to initiate and shape the adaptive immune response.
  - *Follicular dendritic cells*: Located in the germinal centres of lymphoid follicles in the lymph nodes and spleen. These cells bear antigens to Fc portion of IgG and complement proteins, and thus augment secondary antibody responses.

Macrophages

- Process and present antigens to immunocompetent T cells (T cells cannot be activated by soluble antigens and antigen presentation essential for induction of CMI).

- Macrophages are important effector cells in delayed hypersensitivity and humoral immunity.

NK (Natural Killer) Cells

- Constitute10–15% of circulating lymphocytes
- Lack TCR or BCR
- Also called large granular lymphocytes (larger than small lymphocytes and have abundant azurophilic granules)
- Express CD2, CD16 (Fc receptor for IgG) and CD56
- CD 16 aids in type II hypersensitivity (antibody-dependent cell-mediated cytotoxicity or ADCC) by conferring on NK cells the ability to kill infected cells and tumour cells without prior exposure or activation by them. The function of CD 56 is not clear.
- NK cell activity is regulated by stimulatory (NKG2D) and inhibitory (killer cell immunoglobulin-like receptors and CD94 family of lectin receptors) influences.
- Secrete TNF-α, IFN-γ and GMCSF that help NK cells communicate with other cells of immune system.

Innate Lymphoid Cells (ILCs)

- A population of lymphocytes that lack TCRs but generate cytokines similar to T lymphocytes (different subsets produce IFN-γ, IL-5, IL-17, IL-22)
- Their functions include
  - Early defence against infections
  - Elimination of stressed cells
  - Influencing differentiation of T lymphocytes

Q. Enumerate the differences between T lymphocytes and B lymphocytes.

Ans. Differences between T and B lymphocytes are shown in Table 5.1.

TABLE 5.1. Differences between T lymphocytes and B lymphocytes

Features	T cell	B cell
Origin	Stem cells in bone marrow → thymus	Stem cells in bone marrow → secondary lymphoid organs
Life span	• Blasts: several days • Small T cells: months to years	• Blasts: several days • Small B cells: <1 month
Location		
(i) Lymph node	Para/deep cortex	Germinal centre, superficial cortex
(ii) Spleen	Periarteriolar sheath	Germinal centre, red pulp
(iii) Peyer's patches	Perifollicular zone	Follicular centre
Percentage population in		
(i) Blood	80%	20%
(ii) Bone marrow	Rare	Numerous
(iii) Lymph node	85%	15%
Surface markers		
(i) TCR Ag receptor	Present	Absent
(ii) Surface Ig	Absent	Present
(iii) Fc receptor	Absent	Present
(iv) Complement receptor	Absent	Present
(v) CD markers	• T (helper): CD4,3,7,2 • T (suppressor): CD8,3,7,2	CD19,21,23
Functions	(i) CMI via cytotoxic T cells (ii) Delayed hypersensitivity via CD4+ T cells	(i) Precursors of plasma cells (ii) Contribute to humoral immunity by synthesizing specific antibodies (Igs)

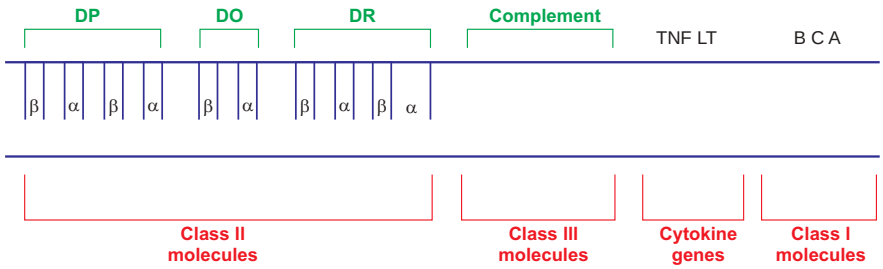


FIGURE 5.3. Components of HLA complex.

Q. Write briefly on HLA complex.

Ans. HLA complex (Fig. 5.3) is a set of multiple genes located on *chromosome 6*. It binds peptide fragments of foreign proteins for presentation to appropriate antigen-presenting cell, and has the following components:

- HLAs corresponding to **MHC class I** (**A**, **B** and **C**) present peptides coming from intracellular proteins, which are produced when the latter are broken down in the proteasomes. These MHC–peptide complexes are recognized by cytotoxic T cells (killer T cells) with the help of the coreceptor CD8. Class I proteins are expressed on the surfaces of nearly all cells.
- HLAs corresponding to **MHC class II** (**DP**, **DQ** and **DR**) present antigens from outside of the cell to T-lymphocytes. These extracellular peptides are taken into the cell with the help of endosomes. The MHC–peptide complexes are recognized by helper T cells with the help of the CD4 coreceptor. These antigens stimulate the multiplication of T-helper cells, which in turn stimulate antibody-producing B cells to produce antibodies to that specific antigen. Self-antigens are suppressed by suppressor T cells. Class II proteins are expressed only on B cells, macrophages and dendritic cells.
- HLAs corresponding to **MHC class III** encode components of the complement system.

Roles of HLA Complex

HLA complex has diverse roles in the human body:

1. Role in disease
   HLA has been found to be associated with a growing number of diseases (Table 5.2). These include
   - Inflammatory diseases like postinfectious arthropathy and ulcerative colitis.
   - Inherited errors of metabolism, like 21-hydroxylase deficiency
   - Autoimmune diseases, like endocrinopathies, ankylosing spondylitis, SLE, myasthenia gravis and Sjögren syndrome. People with certain HLA antigens are more likely to develop certain autoimmune diseases, and HLA typing in autoimmunity is being increasingly used as a tool in diagnosis.
2. Role in graft rejection
   If the immune system recognizes a 'non-self' antigen, it rejects the tissue bearing those antigens. This forms the basis of transplant rejection. Because of the importance of

TABLE 5.2. Association of diseases with HLA

Disease	HLA allele	Disease	HLA allele
Acute anterior uveitis	B27	**Chronic active hepatitis**	DR3
Ankylosing spondylitis	B27	**Primary Sjögren syndrome**	DR3
Postgonococcal arthritis	B27	**Insulin-dependent DM**	DR3, DR4, DR3/DR4
Rheumatoid arthritis	DR4	**21-hydroxylase deficiency**	BW47

HLA in transplantation, the HLA loci are some of the most frequently typed by serology and PCR.

3. Role in cancer

Some HLA-mediated diseases are directly involved in the promotion of cancer; for example, gluten-sensitive enteropathy is associated with increased prevalence of enteropathy-associated T cell lymphoma, and DR3-DQ2 homozygotes comprise the highest risk group.

Q. Differentiate between MHC class I and class II.

Ans. Differences between MHC class I and class II are shown in Table 5.3.

TABLE 5.3. Differences between MHC class I and class II

Features	MHC I	MHC II
Location	Present on all nucleated cells and platelets	Found on antigen-presenting cells–macrophages, dendritic cells and activated T and B cells
Constituted by	Alpha chains (α1, α2, α3) and β2 microglobulin	Alpha chains (α1, α2) and beta chains (β1, β2)
Genes coding region	HLA-A, HLA-B, HLA-C	HLA-D (DP, DQ, DR)
Antigen presentation in association with	CD8+ T cells	CD4+ T cells
Functions	Graft rejection, lysis of virus-infected cells and tumour cells	Graft-versus-host response and immunologic reactions involving CD4+ T cells

Q. Enumerate the characteristics of CD4+ and CD8+ T lymphocytes.

Ans. CD4+ T lymphocyte

- Expressed on 60% of mature T cells (normal CD4 to CD8 ratio is 2:1)
- Called 'helper T cells' because they secrete cytokines, which help B cells in producing antibodies and macrophages in the destruction of phagocytosed microbes
- Also called 'master regulator' of immune system
- Binds to MHC class II
- Helper T cells have three subsets; their features are summarized in Table 5.4

CD8+ T lymphocyte

- Expressed on 30% of mature T cells
- Binds to MHC class I
- Mediates its function primarily as cytotoxic T cell or CTL (directly kill virus-infected cells or tumour cells)

TABLE 5.4. Subsets of helper T cells

Features	T_H 1 cells	T_H 2 cells	T_H 17 cells
Cytokines that induce this subset	IFN-γ and IL-12	IL-4	TGF-β, IL-6, IL-1 and IL-23
Cytokines produced by this subset	IFN-γ	IL-4, 5 and 13	IL-17 and IL-22
Function	Responsible for delayed hypersensitivity, macrophage activation and synthesis of IgG; they provide defence against intracellular microbes.	Responsible for synthesis of other classes of antibodies including IgE; they provide defence against helminthic parasites.	Release IL17, which is a powerful recruiter of neutrophils and monocytes and is important in defence against extracellular bacteria and fungi.
Role in disease	Autoimmune and chronic inflammatory diseases	Allergies	Autoimmune and chronic inflammatory diseases

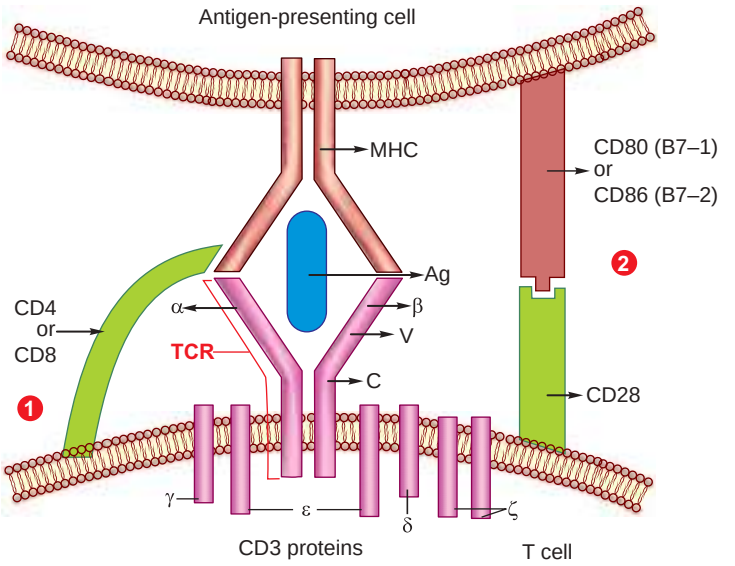


FIGURE 5.4. Antigen recognition by T cells (signals 1 and 2).

Q. Enumerate the steps of antigen recognition by T cells.

Ans. Steps in antigen recognition by T cells (Fig. 5.4):

CD4+ T Cells

- TCR recognizes peptide fragment bound to MHC class II
- CD4 binds to nonpolymorphic portion of MHC class II
- TCR and MHC-bound antigen provides *signal 1* for T cell activation
- CD28+ costimulatory molecules (B7-1 and B7-2) on antigen-presenting cell provide *signal 2*

CD8+ T Cells

- Recognition of antigen in association with MHC class I
- Also need signals 1 and 2 like CD4+ T cells

Q. Differentiate between helper and suppressor T cells.

Ans. Differences between helper and suppressor T cells are shown in Table 5.5.

TABLE 5.5. Differences between helper and suppressor T cells

Features	Helper/inducer T cells	Suppressor/cytotoxic T lymphocytes
Type	• CD4-positive • CD8-negative	• CD8-positive • CD4-negative
Percentage of peripheral T cells	60%	30%
Antigen recognition in association with	HLA class II	HLA class I
Functions	Release lymphokines and activate macrophages and B cells, responsible for delayed hypersensitivity	Cytotoxicity mediated by pore formation and release of granzymes. Also Fas/FasL-dependent killing
Subsets	Two subsets, TH1 and TH2	No subsets

Q. Define hypersensitivity and write in detail on type I hypersensitivity.

Ans. Hypersensitivity is defined as an excessive and potentially harmful reaction to an endogenous or exogenous antigen. It generally occurs in a previously sensitized individual when the balance between the effecter and control immune mechanisms gets disturbed. It is usually associated with inheritance of susceptibility genes (both HLA and non-HLA). HS is classified into four types based on the underlying immune mechanism.

Type I Hypersensitivity

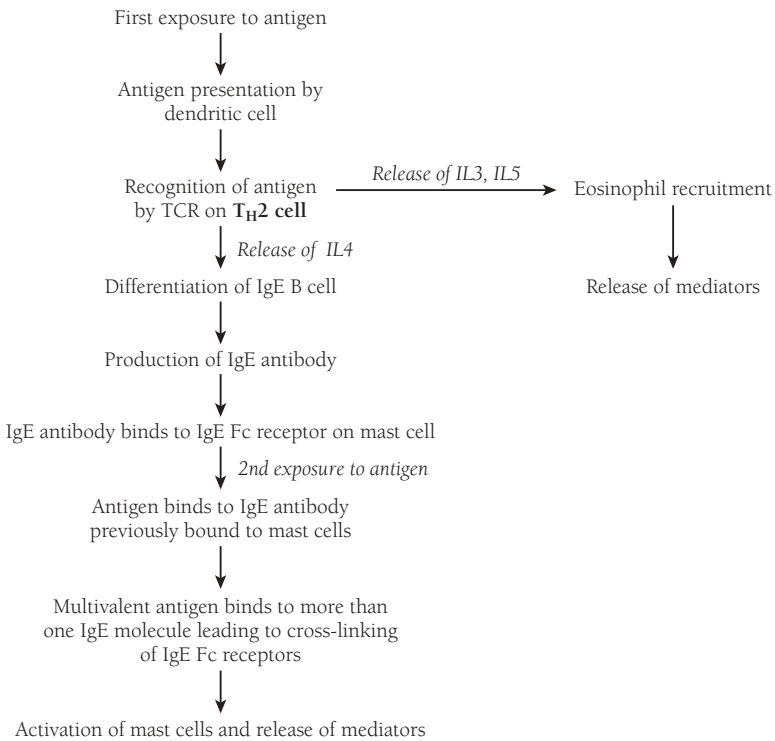
Definition

An immunologic reaction, developing within minutes after combination of an antigen with antibody bound on mast cells or basophils, in already sensitized individuals. Based on the portal of entry type I hypersensitivity is classified into two types:

1. **Local (atopy):** Occurs when the antigen is confined to a particular site. Manifests with skin allergy, hives, nasal and conjunctival discharge, hay fever, bronchial asthma and allergic gastroenteritis. May have two distinct phases, immediate (occurs within minutes of exposure to the antigen and subsides in a few hours) and a late phase (starts 2–24 h later and lasts for days).
2. **Systemic:** Mostly follows parenteral administration (bee venom or an intravenous injection of antisera, hormones, enzymes, drugs, etc.) but can also result from ingestion of the allergen (peanuts). Results in systemic anaphylaxis within minutes of exposure (urticaria, laryngeal oedema, pulmonary bronchoconstriction, vomiting, abdominal cramps and diarrhoea).

Mechanism Underlying Type I Hypersensitivity (Flowchart 5.2)

Immediate hypersensitivity reaction is attributed to excessive T_H2 responses which stimulate IgE production and sensitize and activate eosinophils and mast cells. Mast cells and



FLOWCHART 5.2. Mechanism underlying type I hypersensitivity.

basophils are activated by (a) cross-linking of high-affinity IgE Fc receptors, (b) anaphylatoxins (C3a and C5a) and (c) IL-8. These cells express high-affinity receptors called FcϵRI which are specific for the Fc portion of IgE antibodies and avidly bind them. When in a previously sensitized individual, a mast cell bound to IgE antibodies is exposed to the same antigen; there is activation of the cell and release of powerful mediators.

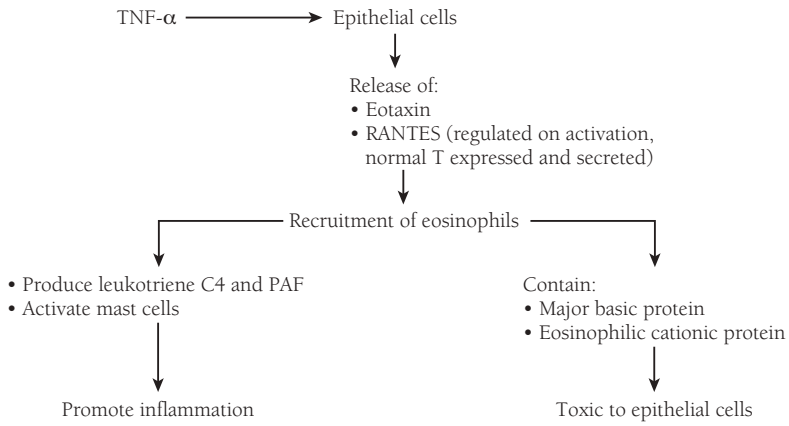
1. **Primary mediators:** These are immediately released already formed stored mediators which induce smooth muscle contraction, increased vascular permeability, increased mucous production (by nasal, bronchial, gastric glands) as well as platelet granule release. They include
   (a) Biogenic amines
      (i) Histamine
      (ii) Adenosine
      (iii) 5-Hydroxytryptamine (5HT)
   (b) Chemotactic mediators
      (i) Eosinophil chemotactic factor (ECF)
      (ii) Neutrophil chemotactic factor (NCF)
   (c) Enzymes
      Contained in granule matrix, eg, proteases (tryptase, chymase) and acid hydrolases
   (d) Proteoglycans
      Heparin and chondroitin sulphate (serve to package and store other mediators in the granules)
2. **Secondary mediators:** These are synthesized de novo and released late.
   (a) Lipid mediators
      (i) Leukotrienes
         - C_4, D_4 (vasoactive and spasmogenic)
         - B_4 (chemotactic for neutrophils, eosinophils and monocytes)
      (ii) Prostaglandin D_2
         - Generated by cyclooxygenase pathway
         - Induce bronchospasm and increased mucous secretion
      (iii) Platelet-activating factor (PAF)
         - Induces platelet activation and bronchospasm
         - Releases histamine
         - Increased vascular permeability
         - Is chemotactic for neutrophils and eosinophils
         - Activates inflammatory cells and causes their aggregation and degranulation
   (b) Cytokines include TNF-α, ILs-1, -3, -4, -5, -6 {T_H2 response}, GMCSF, chemokines, and macrophage inhibitory protein (MIP-1α and β). Cytokines recruit and activate inflammatory cells and mast cells

Q. What are mast cells? How do they contribute to type I hypersensitivity?

Ans. Mast cells are bone marrow-derived cells, widely distributed in tissues near blood vessels and nerves and in subepithelial sites. They contain membrane-bound granules that possess a variety of biological mediators including PAF, histamine, leukotrienes C_4, D_4, E_4, prostaglandins, cytokines, ECF and NCF.

Q. Write briefly on the role of eosinophils in type I hypersensitivity.

Ans. Role of eosinophils in type I hypersensitivity is shown in Flowchart 5.3.



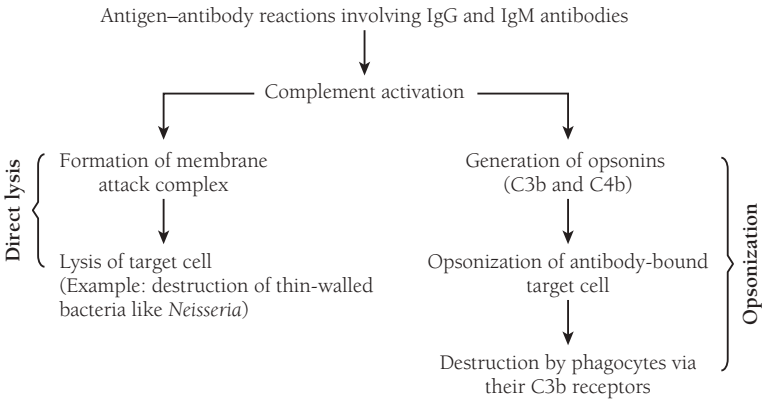
FLOWCHART 5.3. Role of eosinophils in type I hypersensitivity.

Q. Write in detail on type II hypersensitivity.

Ans. Type II hypersensitivity is mediated by antibodies directed towards antigens present on the surface of the cells or other tissue components. These antigens may be intrinsic to cell membrane or exogenous antigens absorbed on the cell surface (eg, a drug metabolite). Reaction occurs when antibodies bind to normal or altered cell surface antigens.

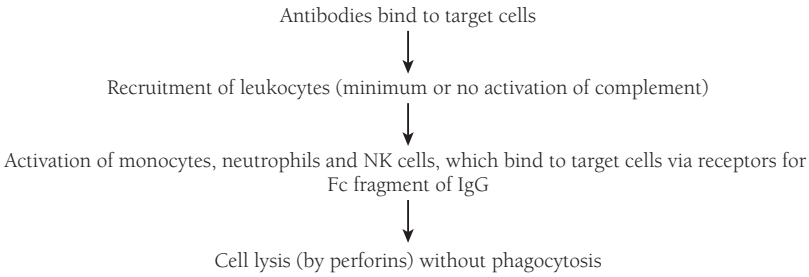
Mechanisms Underlying Type II Hypersensitivity

1. **Opsonization and phagocytosis** (Flowchart 5.4)



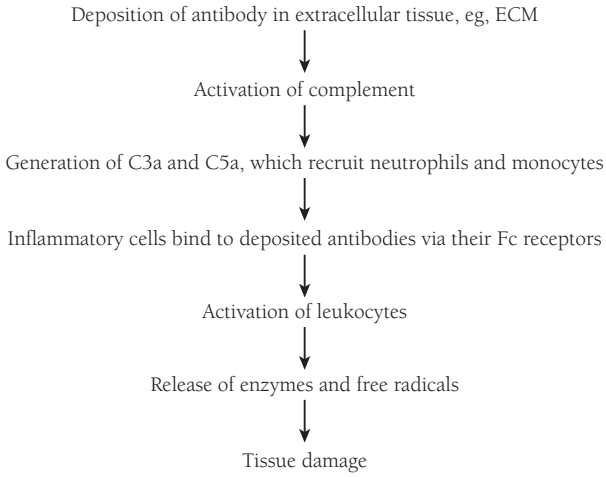
FLOWCHART 5.4. Steps in opsonization and phagocytosis.

2. **ADCC (antibody-dependent cellular cytotoxicity;** Flowchart 5.5**)**: ADCC involves cell lysis without phagocytosis mediated by monocytes, neutrophils and NK cells.
   Examples: Transfusion reactions, autoimmune haemolytic anaemia, erythroblastosis fetalis, agranulocytosis, thrombocytopenia and drug reactions



FLOWCHART 5.5. ADCC.

3. **Complement and Fc receptor-mediated inflammation (Flowchart 5.6):** Deposition of antibody in extracellular tissue initiates an antigen-antibody reaction leading to complement activation. Complement activates inflammatory cells to cause the cell injury.
   Examples: Parasitic infections and tumours



FLOWCHART 5.6. Complement and Fc receptor-mediated inflammation.

4. **Antibody-mediated cellular dysfunction**
   Antibodies against cell surface receptors deregulate function without causing cell injury or inflammation.
   Examples
   - *Myasthenia gravis,* which is due to antibodies against acetylcholine receptors in the motor end plates of skeletal muscle. These antibodies impair neuromuscular transmission and cause muscle weakness.
   - *Pemphigus vulgaris,* which is due to antibodies against desmosomes. These antibodies disrupt the intercellular junction and result in the formation of vesicles.

Q. Write in detail on type III hypersensitivity.

Ans. Type III hypersensitivity is induced by antigen–antibody complexes that produce tissue damage as a result of their capacity to activate the complement system.

Antigen–antibody complexes may be:

1. **Circulating** or **in situ**
2. **Exogenous** (eg, infectious agents and drugs) or **endogenous** (eg, 'nuclear antigens' in SLE, 'immunoglobulins' in reactive arthritis, 'streptococcal cell wall antigens' in acute post-streptococcal glomerulonephritis and 'HBS antigen' in polyarteritis nodosa)
3. **Systemic** (acute serum sickness—prototype of a systemic immune complex disease) or **local** (Arthus reaction—local immune complex disease)

Pathogenesis

- Formation of antigen–antibody complexes (**first phase**)
- Deposition of immune complexes in various tissues (**second phase**)
- Initiation of an inflammatory reaction in dispersed sites throughout the body (**third phase**)

Factors Influencing Deposition of Immune Complexes in Various Tissues

1. Size of immune complexes:
   (a) Large complexes have antibody excess (complex with many free IgG Fc regions) and are readily removed by the mononuclear phagocytic system.

(b) Small- and medium-sized complexes have antigen excess, are cleared less effectively and are the most pathogenic complexes.

2. Functional status of mononuclear phagocytic system (MPS): Intrinsic dysfunction or overload of MPS increases the probability of persistence of immune complexes in circulation and tissue deposition.
3. Charge of immune complex
4. The three-dimensional structure of immune complex
5. Valency of the antigen
6. Affinity of the antigen to tissue components and avidity of antibody
7. Haemodynamic factors

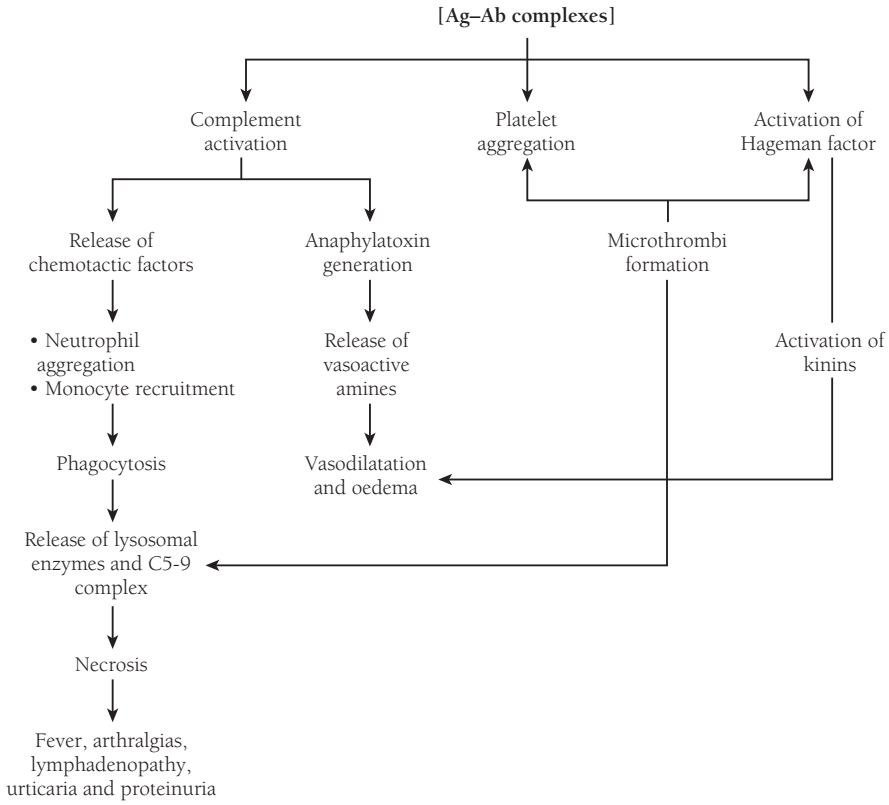
Favoured Sites of Deposition

Renal glomeruli, joints, skin, heart, serosa and small blood vessels.

Morphology of Immune Complex-Mediated Tissue Injury

- Necrotizing vasculitis (fibrinoid necrosis and neutrophils in the vessel wall)
- Swelling and proliferation of endothelial and mesangial cells
- Neutrophilic and monocytic infiltration into glomeruli
- Hypercellular glomeruli
- *Immunofluorescence*: granular lumpy deposits of immunoglobulins and complement
- *Electron microscopy*: electron-dense deposits

Mechanism of Immune Complex-Mediated Tissue Injury (Flowchart 5.7)



FLOWCHART 5.7. Mechanism of immune complex-mediated tissue injury.

Q. What is Arthus reaction?

Ans. Arthus reaction (localized immune complex disease) typically manifests as a localized area of tissue necrosis resulting from *acute immune complex vasculitis involving complement fixing antibodies IgG and IgM.*

- It is usually elicited in the skin.
- Intracutaneous injection into an animal having circulating antibodies against the antigen result in formation of large immune complexes, which precipitate locally and trigger an inflammatory reaction.
- Oedema, haemorrhage and ulceration develop in a few hours and peak in 4–10 h after injection.

Clinical Significance

- *Single large exposure of antigen* causes resolution of the disease due to catabolism of immune complexes (eg, acute serum sickness, acute post-streptococcal glomerulonephritis).
- *Prolonged exposure to antigen* causes chronic disease (eg, SLE).

Q. Write in detail on type IV hypersensitivity.

Ans. Type IV (delayed) hypersensitivity is initiated by specifically sensitized T cells and may be of two types:

1. ***Classic delayed hypersensitivity*** (DTH) mediated by CD4+ T cells
2. ***Direct cell toxicity*** (cytolysis) mediated by CD8+ T cells

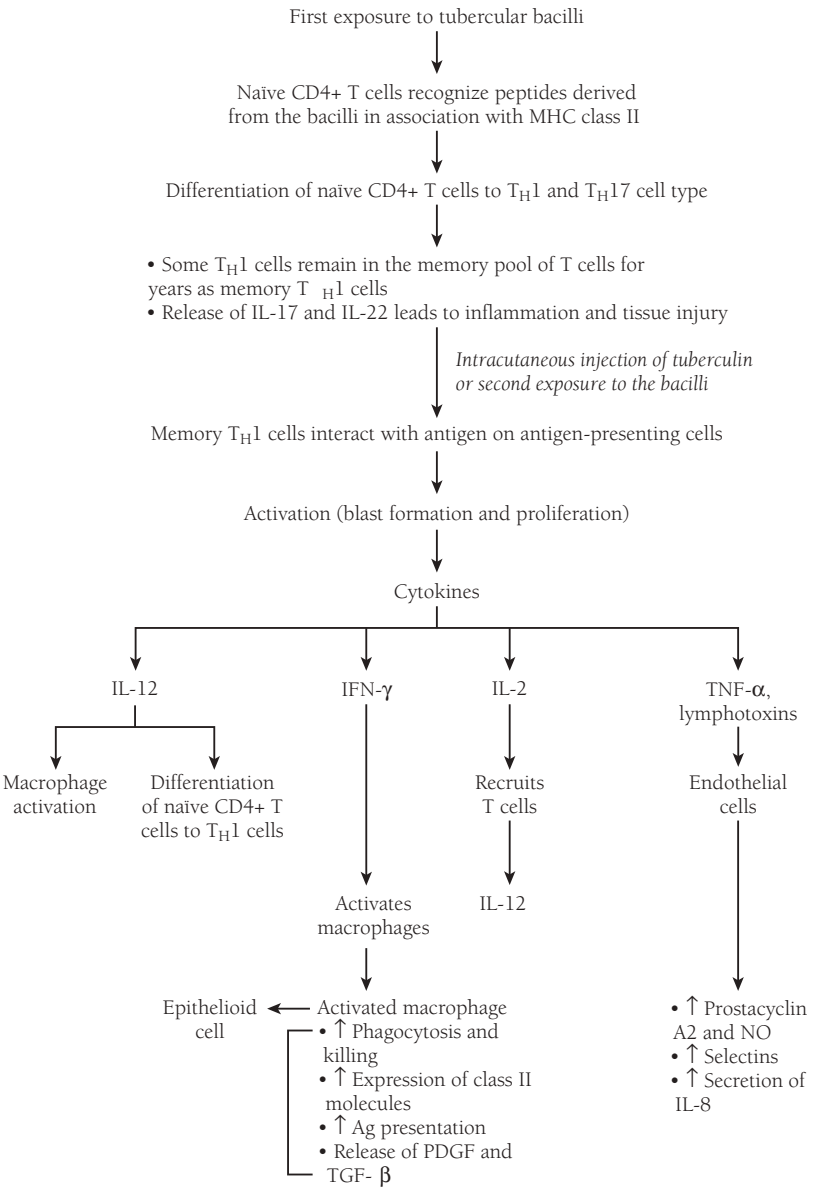
Classic Delayed Hypersensitivity (DTH)

It is the immunologic response to a variety of intracellular microbiologic agents, eg, *M. tuberculosis*, viruses, fungi, protozoa, parasites, as well as conditions like contact dermatitis, type 1 diabetes mellitus, multiple sclerosis and graft rejection. The **classic prototype of DTH is tuberculin reaction** (intracutaneous injection of tuberculin (protein–liposaccharide component of tuberculous bacillus) in a previously sensitized individual resulting in reddening and induration, which starts after 8–12 h and peaks in 24–72 h.

Morphology of DTH

- Accumulation of mononuclear cells around small veins and venules producing perivascular cuffing
- Increased microvascular permeability
- Escape of plasma proteins leading to dermal oedema or deposition of fibrin in interstitium (*induration*)
- Fully developed lesions show endothelial hypertrophy and hyperplasia
- Persistent/nondegradable antigens induce perivascular lymphocytic infiltrate replaced by macrophages in 2–3 weeks. Macrophages are converted into epithelium-like (epithelioid) cells, which aggregate to for granulomas.

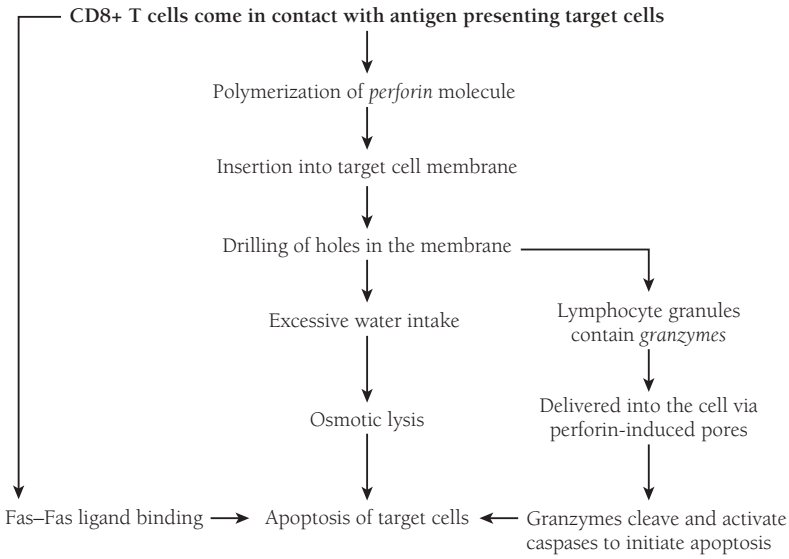
Sequence of Events in Development of DTH (Flowchart 5.8)



FLOWCHART 5.8. Sequence of events in development of DTH.

T Cell-Mediated Cytotoxicity/Cytolysis (Flowchart 5.9):

- Sensitized CD8+ T cells (cytotoxic T lymphocytes or CTLs) kill antigen-bearing target cells and seem to have an important role in:
  - Graft rejection
  - Resistance to viral infections
- Two principal mechanisms of T cell-mediated damage:
  - Perforins- and granzyme-dependent killing: Perforins and granzymes are soluble mediators contained in the lysosome-like granules of CTLs
  - Fas–Fas ligand-dependent killing: Apoptosis of the target cells is caused by Fas–Fas ligand-dependent mechanism.



FLOWCHART 5.9. T cell-mediated cytotoxicity/cytolysis.

Q. Tabulate differences between the different types of hypersensitivity.

Ans. Differences between types I, II, III and IV hypersensitivity are summarized in Table 5.6.

TABLE 5.6. Differences between types I, II, III and IV hypersensitivity

Features	Type I	Type II	Type III	Type IV
Reaction type	Anaphylactic	Cytotoxic	• Serum sickness • Arthus reaction	Delayed hypersensitivity
Cells involved	Mast cells, basophils, eosinophils, neutrophils, monocytes, CD4+ T cells, B cells	Nonsensitized macrophages, NK cells, neutrophils, eosinophils, B cells	Neutrophils, B cells	CD4+ T cells, macrophages, CD8+ T cells
Antibody type	IgE	IgG, IgM	IgG, IgM	None
Chemical mediators	IL-3, 4, 5; vasoactive amines	Complement system	Complement system	Lymphokines, IL-12, IL-2, INF-γ, TGF-β, TNF-α
Antigen presentation by APC	Required	Not required	Required	Required
Pre-sensitization	Required	Not required	Required	Required
Pathogenesis	Formation of IgE and immediate release of mediators to recruit inflammatory cells inducing inflammatory changes	Opsonization and phagocytosis, ADCC, antireceptor antibody type	Formation and deposition of Ag–Ab complexes → activates complement system → neutrophils recruited → release of lysosomal enzymes and other toxic agents	• Sensitized T- lymphocytes mediate release of lymphokines • T cell-mediated cytolysis
Time for onset	Minutes	Hours to days	Hours to days	Hours to days
Examples	Allergic bronchial asthma	Transfusion haemolytic reactions	Glomerulonephritis, rheumatoid arthritis	Transplant rejection

Q. Write briefly on lepromin reaction.

Ans. Lepromin (emulsified, lepromatous tissue rich in lepra bacilli and standardized according to lepra bacilli contents) is injected intradermally and the response is noted.

Two Phases

- **Early reaction of Fernandez** develops in 24–48 h and subsides in 3–5 days. It manifests with erythema and induration. Poorly defined, this reaction has little significance. It is analogous to tuberculin reaction.
- **Late reaction of Mitsuda** starts in 1–2 weeks, reaches its peak in the 4th week and gradually subsides over the next few weeks. It manifests with an indurated skin nodule, which may later ulcerate. Histological sections show infiltration by lymphocytes and formation of epithelioid and giant cells.

Note: This test is used to check CMI status of the individual against lepra bacilli. It is not helpful as a diagnostic test.

Q. Define immunologic tolerance. What are its different types?

Ans. An individual is incapable of developing an immune response to a specific self-antigen and, hence, is capable of living in harmony with one's own cells and tissues. This is called immunologic tolerance. Tolerance is of two types—**central tolerance** and **peripheral tolerance**. **Central tolerance** develops very early in the life of an immune cell. It encounters the self-molecules in the body during development and this initiates a self-destruction pathway, which leads to the death of the cell before it attains maturity. This process, called clonal deletion, helps ensure that 'self-reactive' T cells and B cells, that could develop the ability to destroy the body's own cells, do not mature and attack healthy tissues.

In **peripheral tolerance**, the body's immune cells might recognize a self-molecule but do not build up an immune response to it (switch off) because some of the chemical signals required to activate the T or B cell are absent. This is labelled clonal anergy. A special class of regulatory T cells that inhibits helper or cytotoxic T cell is involved in the development of peripheral tolerance.

1. **Mechanisms of development of central tolerance (clonal elimination or deletion):**
   (a) Central tolerance develops during lymphocyte development and operates in the thymus and bone marrow.
   (b) Here, T and B lymphocytes that recognize self-antigens are deleted before they mature into fully immunocompetent cells, preventing autoimmunity.
   (c) Self-antigens are present in both organs due to endogenous expression within the organ and importation of antigen due to circulation from peripheral sites. In the case of T cell central tolerance, additional sources of antigen are made available in the thymus by the action of the transcription factor AIRE (**autoimmune regulator**).
   (d) **Positive selection** occurs first when näive T cells are exposed to antigens in the thymus. T cells, which have receptors with sufficient affinity for self-MHC molecules are selected. Other cells that do not show sufficient affinity to self-antigens will undergo a deletion process known as **death by neglect,** which involves apoptosis of the cells. This does not occur in B cells.
   (e) **Negative selection** of T cells with a very high affinity of self-MHC molecules is induced to anergy or lineage divergence to form T-regulatory cells.
2. **Mechanisms of development of peripheral tolerance**
   (a) **Clonal anergy**
      - Prolonged or irreversible inactivation of lymphocytes is labelled anergy.
      - Activation of antigen-specific T cells requires two signals:
        - Recognition of peptide antigen in association with MHC molecule on the surface of antigen-presenting cell
        - Second set of costimulatory signals provided by antigen-presenting cells
      - Certain T cell-associated molecules such as CD28 must bind to their ligands B7-1 and B7-2.
      - If the antigen-presenting cell does not bear a CD28 ligand, a negative signal is generated to induce anergy.

(b) **Peripheral suppression by T cells**
- A population of T cells is called 'regulatory T cells'. These cells have the ability to down regulate function of autoreactive T cells. This is mediated by secretion of cytokines.
- CD4+ T cells of T_H2 type are the best known 'regulatory cells' and are thought to mediate their action via cytokines like IL4, IL10 and TGF β.

(c) **Clonal deletion by activation-induced cell death**
- CD4+ T cells that recognize self-antigens may receive signals that promote their death by apoptosis.
- Lymphocytes express Fas (CD95), a member of TNF receptor family.
- Fas ligand (Fas L) is a member protein that is structurally homologous to the cytokine TNF, and is expressed mainly on activated T lymphocytes.
- Engagement of Fas by Fas L induces apoptosis of activated T cells and may cause peripheral deletion of autoreactive T cells.
- Self-reactive B cells are also deleted by Fas L on T cells due to engaging of Fas on B cells.

(d) **Antigen sequestration**

Some antigens are hidden from the immune system because the tissues in which these antigens are located do not communicate with the blood and lymph, eg, testis, eye and brain.

Q. Differentiate between central and peripheral tolerance.

Ans. Differences between central and peripheral tolerance are enlisted in Table 5.7.

TABLE 5.7. Differences between central and peripheral tolerance

Features	Central tolerance	Peripheral tolerance
Origin	Thymus/bone marrow	Peripheral tissue
Mechanism	Clonal deletion of self-T/B cells	Clonal deletion, clonal anergy, peripheral suppression by T cells
Role in autoimmune diseases	Failure may not result in autoimmune diseases	Failure usually results in autoimmune diseases

Q. Write in detail on mechanism of development of autoimmunity.

Ans. Development of autoimmunity is related to:

- **Susceptibility genes** which influence the maintenance of self-tolerance
- **Environmental triggers,** particularly **infections,** which promote the activation of self-reactive lymphocytes

Mechanisms of Development of Autoimmunity

1. **Breakdown of T cell anergy**
   (a) Autoreactive T cells that escape clonal deletion are rendered anergic when they encounter self-antigens expressed on costimulator deficient antigen-presenting cells (APCs).
   (b) Anergy is broken if APCs are induced to express costimulatory molecules (induction may occur consequent to infections, tissue necrosis, inflammation, etc.).
2. **Failure of activation-induced cell death:** Defects (congenital or acquired) in the Fas–Fas ligand system (responsible for apoptosis) may lead to persistence and proliferation of autoreactive T cells in the peripheral tissues.

3. **Failure of T cell-mediated suppression:** Loss of regulatory/suppressor T cells that can limit the function of autoreactive T and B cells.
4. **Molecular mimicry**
   (a) Some infective agents share epitopes with self-antigens.
   (b) Immune response against such microbes produces a tissue damaging reaction, eg, rheumatic heart disease: cross-reaction between antibodies to streptococcal M protein and cardiac glycoproteins.
5. **Polyclonal lymphocyte activation**
   (a) Several microorganisms and their products (eg, bacterial lipopolysaccharides or endotoxins) are capable of inducing polyclonal B cell activation or CD4+ T cells activation in an antigen-independent manner. Because they stimulate all T cells that are associated with a set of V TCRS, they are called super antigens (SAgs).
   (b) They do so by binding to MHC class II on APC and β chains on TCR outside the antigen-binding groove.
   (c) This causes a massive immune response that is not specific to any particular epitope on the SAg.
   (d) Among T cells activated by super antigens, some may be reactive to self-antigens leading to autoimmunity.
   (e) SAgs produce an immune response that is effectively useless. Microbes produce SAgs as a defence mechanism to aid them in evading the immune system.
6. **Release of sequestered antigens (anatomic sequestration):** Any self-antigen that is completely sequestered during development is likely to be viewed as *foreign when introduced in the circulation*, eg, spermatozoa {post-traumatic orchitis) and ocular antigens (uveitis).
7. **Exposure of cryptic self-antigens and epitope spreading (molecular sequestration):** A large number of self-determinants are not readily recognized by the immune system, and hence, T cells specific for such 'cryptic' self-epitopes are not deleted.

Evidence Implicating Genetic Factors in Development of Autoimmunity

- Familial clustering
- Linkage of autoimmune diseases with HLA (Table 5.8)

TABLE 5.8. Linkage of autoimmune diseases with HLA

S. No.	Disease	HLA allele
1.	Rheumatoid arthritis anti-cyclic citrullinated peptides (CCP) antibody positive.	DR4
2.	Type 1 diabetes	DR3, DR4, DQB1 position-β 57
3.	Multiple sclerosis	DR15
4.	Systemic lupus erythematosus	DR3, DR8, DR15
5.	Ankylosing spondylitis	B27
6.	Celiac disease	DQ2, DQ8

- Induction of autoimmune diseases in HLA-B27 transgenic rats
- Linkage of autoimmune diseases with non-MHC genes, which may be either disease specific or associated with the multiple disorders, eg, polymorphisms in **PTPN22**, polymorphisms in **NOD2** and the genes coding for **IL-2 receptor** and **IL-23 receptor** (Table 5.9)

TABLE 5.9. Linkage of autoimmune diseases with non-MHC genes

Gene involved	Disease	Function
PTPN22	RA, IBD	Protein tyrosine phosphatase; affects signalling in lymphocytes
IL2RA	MS	α-chain of the receptor for IL-2; important in growth and survival of activated and regulatory T cells
IL23R	IBD, PS, AS	Receptor for TH17 inducing cytokine IL-23; may affect differentiation of CD4+ cells into pathogenic TH17 effector cells
CTLA4	RA	Terminates activation and promotion of regulatory T cells; inhibit T cell responses and interfere with self-tolerance
NOD2	IBD	May control resistance to gut commensals
ATG16	IBD	May be involved in autophagy of microbes

Q. Classify autoimmune diseases.

Ans. Classification of autoimmune diseases is given in Table 5.10.

TABLE 5.10. Classification of autoimmune diseases

Organ specific	Systemic
• Autoimmune haemolytic anaemia • Atrophic gastritis (pernicious anaemia) • Multiple sclerosis (MS) • Good pasture syndrome • Insulin-dependent diabetes mellitus • Graves disease • Hashimoto thyroiditis	• Rheumatoid arthritis • Sjögren syndrome • Systemic lupus erythematosus (SLE)

Q. Describe the aetiopathogenesis and clinicopathological features of systemic lupus erythematosus (SLE).

Ans. SLE is a classical prototype of a multisystem disease of autoimmune origin.

Clinical Features

- Chronic, remitting, relapsing commonly febrile illness characterized by injury to skin, joints, kidneys and serosal membranes
- Females are more commonly affected than males
- May be acute or insidious in onset; usually arises in the second or third decade (no age exempt)

Aetiology

- Fundamental defect in regulatory mechanisms that sustain self-tolerance
- Characterized by presence of antibodies to:
  1. Nuclear antigens
  2. Cytoplasmic antigens
  3. Cell surface antigens
  4. Antigens of blood elements
- Antinuclear antibodies (ANAs) include
  1. Antibodies to DNA
  2. Antibodies to histones
  3. Antibodies to nonhistone proteins bound to RNA
  4. Antibodies to nucleolar antigens

- Most reliable technique to demonstrate ANAs is indirect immunofluorescence (IF) which shows four basic patterns, namely:
  1. Homogenous/diffuse nuclear staining: Antibodies to chromatin, histones and double stranded (ds) DNA
  2. Rim or peripheral staining patterns: Antibodies to ds DNA
  3. Speckled pattern: Most common and least specific pattern. IF shows uniform- and variable-sized specks, which indicate presence of antibodies to sm (Smith) antigen, RNP (ribonucleoprotein), SS-A and SS-B
  4. Nucleolar pattern: Most commonly seen in systemic sclerosis. IF shows discrete spots in the nucleus which indicate antibodies to nucleolar RNA.
- The fluorescence patterns are not absolutely specific for the type of antibody as there can be more than one antibody or a combination of patterns.
- Antibodies to Sm antigen and ds DNA are however virtually diagnostic of SLE.
- Correlation between presence of certain ANAs and clinical manifestations is noted, eg, high titers of ds DNA antibodies are found to be associated with active renal disease.
- Antiphospholipid (AP) antibodies (antibodies against plasma proteins complexed to phospholipids, eg, prothrombin, annexin V, β2 glycoprotein 1, proteins C and S) as well as antibodies against RBCs, platelets and lymphocytes may also be seen in SLE.

Pathogenesis of SLE

Pathogenesis of SLE is thought to be multifactorial.

- Genetic factors
  - Increased risk in family members and concordance in monozygotic twins noted
  - MHC genes are thought to regulate the production of autoantibodies (specific polymorphisms of HLA-DQ are linked to the production of anti-ds DNA, anti-Sm and AP antibodies)
  - Non-MHC genes may also contribute
  - Lupus patients may have inherited deficiency of early complement components; eg, C2 (lack of complement impairs removal of circulating immune complexes)
  - The genome-wide association studies have indicated the involvement of several genetic loci. These loci encode proteins which are responsible for lymphocyte activation and cytokine (IFN) responses.
- Environmental factors
  - Drugs such as hydralazine and procainamide are known to induce an SLE-like response.
  - Ultraviolet light may bring about changes in DNA such that it becomes immunogenic.
  - Sex hormones are thought to be involved in the pathogenesis (since females are affected more than males).
- Immunologic factors
  - Susceptibility genes interfere with normally existing self-tolerance. Environmental insults induce apoptosis and defective clearance of the nuclei of the apoptotic bodies which in turn increases the burden of nuclear antigens.
  - Self-nucleic acids may mimic their microbial counterparts to activate TLRs which send signals to B cells specific for nuclear antigens as well as dendritic cells resulting in production of antinuclear antibodies. Dendritic cells produce type 1 IFN which stimulates B cells.
  - Tissue damaging antibodies are driven against self-antigens (results from an antigen-specific helper T cell-dependent B cell response). The lesions of SLE are mainly caused by immune complexes (type III HS).

Classification of SLE is given in Table 5.11.
4/11 criteria should be present for diagnosis of SLE.

Morphological Features of SLE

Most characteristic lesions result from deposition of immune complexes in the kidneys, connective tissues and skin.

TABLE 5.11. Year 1997 revised criteria for the classification of SLE

1.	Malar rash	Fixed erythema, flat or raised over the malar eminences
2.	Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging
3.	Photosensitivity	Skin rash due to exposure to UV light
4.	Oral ulcers	Oral or nasopharyngeal ulceration
5.	Arthritis	Nonerosive arthritis involving two or more peripheral joints causing tenderness, swelling and effusion
6.	Serositis	Pleuritis (history of pleuritic pain or rub or effusion), pericarditis (ECG documentation or pericardial rub or effusion)
7.	Renal disorder	Persistent proteinuria (>0.5g/dl) and cellular casts (RBC, haemoglobin, granular, tubular or mixed)
8.	Neurological disorder	Seizures and psychosis (unexplained)
9.	Haematological disorder	Haemolytic anaemia with reticulocytosis, leucopenia ($<4x10^9$ cells/L), lymphopenia ($<1.5x10^9$ cells/L) and thrombocytopenia ($<100x10^9$ cells/L)
10.	Immunologic disorder	Anti-ds DNA, anti-Sm antibody; positive findings of antiphospholipid syndrome (increased IgM or IgG anticardiolipin antibodies, positive test for lupus anticoagulant, false positive serologic test for syphilis confirmed by negative TPI or FTABS)
11.	Antinuclear antibodies	Positive antinuclear antibodies (in the absence of drugs known to be associated with drug-induced SLE)

Kidneys

- Light microscopic involvement is seen in 60–70% cases; whereas, immunofluorescence (IF) and electron microscopic (EM) changes are seen in most cases.
- Five morphological patterns are recognized based on WHO morphologic criteria:
  1. Class I: Rare; no changes seen on light microscopy; however, IF or EM can identify immune complex deposition in the mesangium.
  2. Class II: Also called mesangial lupus glomerulonephritis, this comprises 20% of all cases and manifests with minimal clinical symptoms. It is morphologically characterized by:
     (a) Increased intercapillary mesangial matrix and cells
     (b) Granular mesangial deposits of immunoglobulins and complement on IF
  3. Class III: Also called focal proliferative glomerulonephritis, this manifests with mild to moderate haematuria and proteinuria. It is morphologically characterized by:
     (a) Involvement of less than 50% of all glomeruli.
     (b) Swelling and proliferation of endothelial and mesangial cells.
     (c) Neutrophilic infiltrate; fibrinoid deposits, and intercapillary thrombi. Extracapillary proliferation with crescent formation may also be seen.
  4. Class IV: Also called diffuse proliferative glomerulonephritis, this is overtly symptomatic and shows the following morphological features:
     (a) More than 50% glomeruli are affected.
     (b) Involvement of the entire glomerulus is labelled 'global' (IV-G) glomerulonephritis and part of the glomerulus is labelled 'segmental' (IV-S) glomerulonephritis
     (c) There is proliferation of endothelial, mesangial and epithelial cells.
     (d) Epithelial crescents may be seen in Bowman's space.
     (e) Also present are fibrinoid necrosis and hyaline thrombi in glomeruli.
  5. Class V: Also called membranous glomerulonephritis. It comprises 15% of all cases and manifests with severe proteinuria with nephrotic syndrome. Main light microscopic change is widespread thickening of capillary walls.
  6. Class VI: Advanced sclerosing lupus nephritis represents end-stage renal disease wherein >90% glomeruli are sclerosed.
- The immune complexes may be deposited in the basement membrane, mesangium, subepithelial zone (between basement membrane and visceral epithelial cells, as in membranous glomerulonephritis) and subendothelial zone (between basement membrane and endothelium). When extensive, subendothelial deposits create a peculiar thickening of the capillary wall called '**wire loop lesions**'.

- Changes in interstitium and tubules may be seen in cases with diffuse involvement
- Prominent changes in other organs include:
  (a) Libman–Sacks endocarditis (nonbacterial verrucous endocarditis)
  (b) Capsular thickening, follicular hyperplasia, increased plasma cells and thickening of penicilliary arteries (onion skinning) in spleen
  (c) Pleuritis, pleural effusion, alveolar injury in the form of oedema and haemorrhage and chronic interstitial fibrosis in lungs

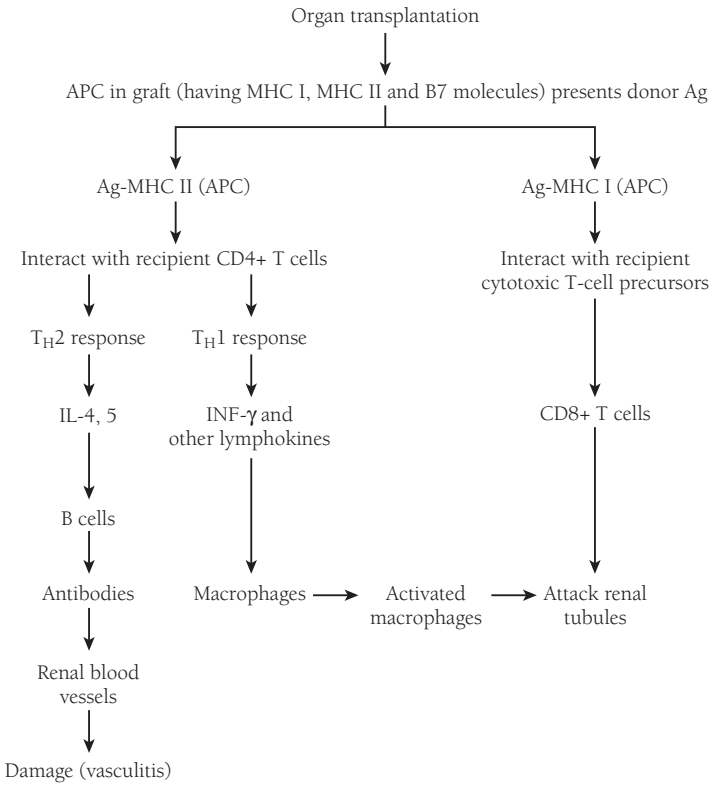
Q. Define transplant rejection. Describe the pathogenesis, clinical features and morphology of acute and chronic rejection.

Ans. Transplant rejection is defined as recognition by the host of the grafted tissue as foreign. Rejection is a complex process in which both CMI and circulating antibodies play a role.

T cell-mediated reactions (cellular rejection):

Occurs due to cytotoxic CD8+ T lymphocytes-mediated killing of grafted cells or delayed hypersensitivity, triggered by activated CD4+ T helper cells, and is mediated by two main pathways:

1. **Direct pathway** (Flowchart 5.10)



FLOWCHART 5.10. Direct pathway of cellular rejection.

2. **Indirect pathway**
   (a) Recipient T lymphocytes recognize antigens of the graft donor after they are presented by the recipient's own antigen-presenting cells.
   (b) Uptake and processing of MHC molecules shed from the grafted organ by host antigen-presenting cells.

Antibody-mediated reactions

These are due to preformed antibodies, (eg, hyperacute rejection)

Morphology of rejection

(a) *Hyperacute rejection*
   (i) Occurs minutes or hours after transplantation and leads to a cyanotic, mottled and flaccid kidney excreting a few drops of bloody urine.
   (ii) There is a rapid accumulation of neutrophils within arterioles, glomeruli and peritubular capillaries along with deposits of immunoglobulins and complement in the vessel wall. EM shows endothelial injury with fibrin-plated thrombi.

(b) *Acute rejection*
   (i) Occurs days, months or even years after transplant.
   (ii) Both cellular and humoral responses involved.

   Features of acute cellular rejection:
   - Increased serum creatinine
   - Clinical signs of renal failure
   - Extensive interstitial mononuclear infiltrate, oedema and haemorrhage
   - Mononuclear cells in the glomerular and peritubular capillaries, which may invade tubules to induce tubular necrosis
   - Vascular endothelial injury mediated by CD8+ T cells

   Features of acute humoral rejection:
   - Mediated primarily by the antidonor antibodies
   - Characterized by the necrotizing vasculitis, endothelial cell necrosis, neutrophilic infiltrate and deposition of immunoglobulins along with complement and fibrin

(c) *Chronic rejection*
   (i) Progressive rise of serum creatinine over a period of 4–5 months is the hallmark.
   (ii) It is dominated by vascular changes (dense intimal fibrosis), interstitial fibrosis, glomerular loss, tubular atrophy, shrinkage of renal parenchyma, interstitial infiltrate of plasma cells and eosinophils.

Q. Differentiate between acute and chronic transplant rejection.

Ans. Differences between acute and chronic rejection are enlisted in Table 5.12.

TABLE 5.12. Differences between acute and chronic rejection

Features	Acute rejection	Chronic rejection
Onset	Occurs within days of transplant	Occurs over months to years after a transplant
Components	Acute cellular and humoral (antibody-mediated) rejection	Cell-mediated rejection characterized by progressive organ dysfunction
Mechanism	Interstitial mononuclear infiltration by CD4+ and CD8+ T cells, endothelial injury and antibody-mediated damage	Mononuclear infiltrate with numerous plasma cells and eosinophils
Morphology	Damaged tubular epithelium, rejection vasculitis (necrotizing vasculitis, thrombosis or intimal thickening)	Arterioles show dense intimal fibrosis leading to parenchymal ischaemic injury

Q. Write briefly on transplantation of hematopoietic cells.

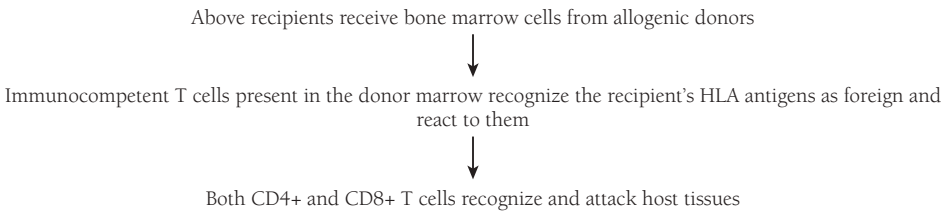
Ans. Indications

- Haematological malignancies
- Nonhaematological cancers
- Aplastic anaemia
- Immunodeficiency states
- Transplantation of genetically engineered hematopoietic stem cells useful for somatic cell gene therapy

Problems With Bone Marrow Transplantation (BMT)

- GVHD (graft-versus-host disease)
- Transplant rejection
- Immunodeficiency

GVHD occurs in any situation in which immunologically competent cells or their precursors are transplanted into immunologically crippled recipients and the transferred cells recognize alloantigens in the host, eg, BMT, transfusion of solid organs rich in lymphoid cells, and transfusion of nonirradiated blood (Flowchart 5.11).



FLOWCHART 5.11. Mechanism of GVHD.

GVHD may be

(a) Acute
  (i) Occurs days to weeks after transplant
  (ii) Causes considerable damage mediated by cytokines without infiltration of lymphocytes
  (iii) Any organ may be affected
  (iv) Major clinical manifestations result from involvement of immune system, epithelia of the skin, liver and intestines, eg, generalized rash with desquamation, mucosal ulceration with bloody diarrhoea and jaundice

(b) Chronic
  (i) May follow acute GVHD or occur insidiously
  (ii) Characterized by extensive cutaneous injury, destruction of skin appendages and fibrosis of dermis (differential systemic sclerosis)
  (iii) Manifests with chronic liver damage with cholestasis, oesophageal strictures and life-threatening infections (due to involution of thymus and depletion of lymphocytes in lymph nodes)

Q. Describe the physical and chemical nature of amyloid.

Ans. Amyloid is an amorphous, eosinophilic, pathologic, proteinaceous substance deposited in between cells or extracellularly.

- First described by Rokitansky in 1842; it was named 'amyloid' by Virchow. It is not a distinct entity but a group of diseases having in common deposition of similar appearing proteins constituted by insoluble abnormal fibrils that injure tissue.
- The fibrils are formed by the aggregation of misfolded, abnormally soluble proteins which bind to various proteoglycans and glycosaminoglycans (heparin and dermatan sulphate and serum amyloid P protein or SAP). Amyloid was so named because the charged sugar groups in the adsorbed proteins resulted in a staining pattern similar to amylase, it was however later found to be unrelated to starch.

Physical Nature of Amyloid

The main physical constituents of amyloid are nonbranching fibrils of indefinite length and a diameter of 7.5–10 nm, which on X-ray crystallography and infrared spectroscopy show a cross-β-pleated sheet conformation

Chemical Nature of Amyloid

- Amyloid is composed of 95% fibril proteins, 5% P (pentagonal) component and other glycoproteins
- There are 23 biochemically different fibril proteins, of which the most common ones are:

AL (amyloid light chain) protein
- The precursor protein is a clonal immunoglobulin light chain or light chain fragment, derived from plasma cells.
- Most AL are composed of lambda light chains; some have kappa chains.
- AL amyloidosis is associated with monoclonal B cell proliferations. It is labelled 'primary amyloidosis' because it is not associated with any primary disease and its clinical manifestations are due to effects of amyloid deposition. A large number of AL amyloidoses have marrow plasmacytosis.

AA (amyloid-associated) protein
- It has a molecular weight of 8500 kDa and 76 amino acid residues
- Derived from precursor SAA (serum amyloid-associated) protein; it is an acute phase reactant that circulates in the serum bound to high-density lipoprotein, HDL-3.
- AA protein deposits are associated with 'secondary amyloidosis' which occurs secondary to inflammatory conditions like tuberculosis, bronchiectasis, chronic osteomyelitis, autoimmune diseases and heroin abuse.

Transthyretin (TTR)
- TTR is a normal serum protein synthesized in the liver and choroid plexus that binds and transports thyroxine and retinal protein
- A mutant form is deposited in familial amyloidotic polyneuropathies and in the hearts of aged individuals (senile systemic amyloidosis)

β2 microglobulin
- β2 microglobulin is a component of MHC Class I
- It is a normal serum protein whose levels increase in patients on long-term haemodialysis

*A*β protein
- *A*β protein is a 4000 Da protein derived from amyloid
- It constitutes cerebral plaques in Alzheimer disease and is derived by proteolysis from a glycoprotein called amyloid precursor protein

Q. Classify amyloidosis. Describe the aetiology and clinicopathological features of the same.

Ans. Classification of amyloidosis (Table 5.13**):** The classification of amyloidosis is based on the tissue distribution of amyloid deposits (**local or systemic amyloidosis**), the absence or presence of pre-existing disease (**primary or secondary amyloidosis**) and the chemical type of amyloid protein fibril.

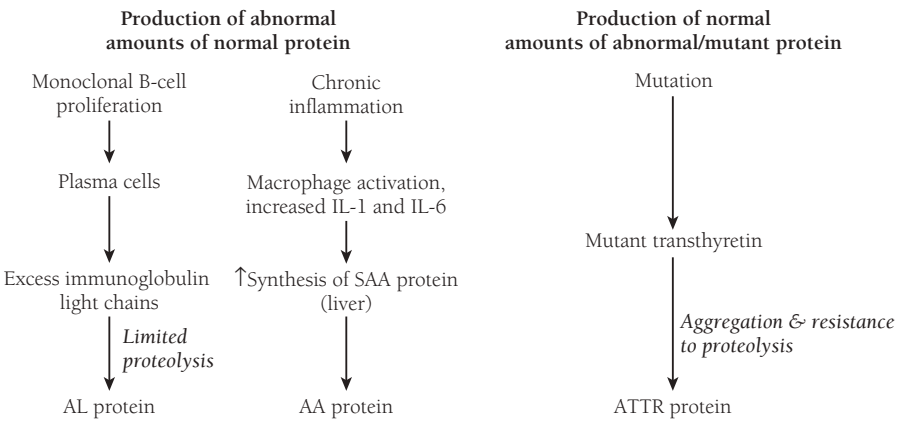
TABLE 5.13. Classification of amyloidosis

Clinicopathological category	Associated conditions	Major fibril protein	Precursor protein
Systemic			
Primary amyloidosis	Multiple myeloma and other monoclonal B cell proliferations	AL	Ig light chains
Secondary amyloidosis	Chronic inflammatory conditions	AA	SAA
Haemodialysis-related amyloidosis	Chronic kidney disease	$A\beta_2m$	β2 microglobulin
Hereditary amyloidosis	—	AA	SAA

TABLE 5.13. Classification of amyloidosis—cont'd

Clinicopathological category	Associated conditions	Major fibril protein	Precursor protein
Familial Mediterranean fever	—	ATTR	Transthyretin
Familial amyloidotic neuropathies	—	ATTR	Transthyretin
Senile systemic amyloidosis	—	ATTR	Transthyretin
Localized amyloidosis			
Senile cerebral	Alzheimer disease	Aβ	APP
Endocrine			
Medullary carcinoma thyroid	—	A Cal	Calcitonin
Islet of Langerhans	Type II disease		Islet amyloid peptide
Isolated atrial amyloidosis	—	AANF	Arial natriuretic factor
Prion disease	Various prion diseases of the CNS	Misfolded prion proteins (PrP^{sc})	Normal prion protein

Pathogenesis of Amyloidosis (Flowchart 5.12)



FLOWCHART 5.12. Pathogenesis of amyloidosis.

There are Two Types of Amyloid Proteins:

1. **Misfolded proteins** (*production of abnormal amounts of normal protein* which is unstable, self-associates to form oligomers and fibrils, called misfolded proteins).
2. **Mutant proteins** (*production of normal amounts of abnormal/mutant protein* which is structurally unstable, prone to misfolding and subsequent aggregation). Abnormally folded proteins get deposited in extracellular tissue as fibrils and disrupt normal tissue by causing pressure atrophy of adjacent cells and vascular narrowing; the latter leading to ischaemia.

Morphology of Amyloidosis

- **Primary amyloidosis:** Affects kidneys, liver, spleen, lymph nodes, adrenal and thyroid
- **Secondary amyloidosis:** Affects heart, kidneys, GIT, peripheral nerves, skin and tongue

Gross Features

- Affected organs are enlarged, firm and waxy.
- Painting cut-surface with iodine imparts a yellow colour, which changes to bluish-violet after application of sulphuric acid.

Staining Characteristics

- **Congo red**
  - Ordinary light-pink or red colour
  - Polarized light-apple green birefringence (due to cross-β-pleated configuration)
- **Metachromatic stains (Rosaniline dyes):** Examples are methyl violet and crystal violet. Amyloid takes up a rose pink colour with these dyes.
- **Fluorescent stains of Thioflavin T and S:** In ultraviolet light, amyloid fluoresces yellow.
- **Immunohistochemistry:** Anti-AA and anti-lambda, anti-kappa antibodies can be used to differentiate between different types of amyloid.
- **Toluidine blue:** Blue colour in ordinary light and dark red, birefringence under polarized microscopy.
- **Alcian blue:** Blue-green colour.
- **PAS (periodic acid-Schiff) and H&E stains:** Pink colour.

Histopathology

Kidneys (most common and most serious form of organ involvement)
Gross features

- Kidneys are normal or enlarged in early stage and shrunken or contracted in late stage (amyloid deposition causes vascular narrowing leading to shrinking of the organ)

Microscopic features

- Primarily glomerular deposits
- Subtle thickening of mesangial matrix due to mesangial deposits
- Uneven widening of basement membrane of glomerular capillaries leading to capillary narrowing due to basement membrane deposits
- Distortion of glomerular vascular tuft due to confluent masses of or interlacing broad ribbons of amyloid

Spleen

***Gross features*:**

Moderate to marked splenomegaly

Microscopic features:

There are two patterns of deposition -

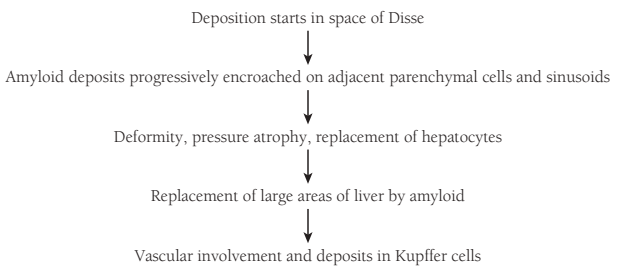
1. Sago spleen: Deposits largely limited to splenic follicles; entire follicle replaced by amyloid, leading to Tapioca-like granules
2. Lardaceous spleen: Sparing of follicles; involvement of walls of splenic sinuses and connective tissue framework of red pulp, leading to large map like areas

Liver

***Gross features*:**

Moderate to marked hepatomegaly

Microscopic features (Flowchart 5.13):



FLOWCHART 5.13. Evolution of morphological changes in hepatic amyloidosis.

Heart

- Involved in systemic amyloidosis (immunocyte dyscrasias) and appears enlarged and firm
- Focal subendocardial accumulation within the myocardium with pressure atrophy of muscle fibres may induce ECG abnormalities

Other organs

- **Adrenals:** Demonstrate deposits along basement membrane of cortical cells in zona glomerulosa
- **GIT:** Early lesions affect blood vessels but later submucosa, muscularis and sub-serosa can be affected
- **Tongue:** Undergoes enlargement (pseudo tumour formation or macroglossia)
- **Respiratory tract:** Shows diffuse involvement of large and small bronchioles

Clinical Features

- **Early, nonspecific:** Weakness, loss of weight and light-headedness
- **Renal involvement:** Nephrotic syndrome (proteinuria), renal failure and uraemia
- **Cardiac involvement:** Conduction disturbances, arrhythmias, restrictive cardiomyopathy, congestive cardiac failure and constrictive pericarditis
- **Tongue involvement:** Hampers speech and swallowing
- **GIT involvement:** Diarrhoea, malabsorption and digestive disturbances

Diagnosis

Depends on demonstration of amyloid by:

- **FNAC** of abdominal fat
- **Biopsy** of kidney (in case of renal involvement), rectum or gingiva

Investigations for Primary Amyloidosis

- Serum/urine electrophoresis
- Immunoelectrophoresis
- Bone marrow aspiration

Q. Differentiate between primary and secondary amyloidosis.

Ans. Contrasting features of primary and secondary amyloidosis are enlisted in Table 5.14.

Q. Write briefly on primary or congenital immune deficiency diseases.

Ans. Caused by mutations in genes involved in lymphocyte maturation or function.

TABLE 5.14. Contrasting features of primary and secondary amyloidosis

Features	Primary amyloid	Secondary amyloid
Biochemical composition	AL (light chain proteins); lambda chains more than kappa	AA, derived from larger precursor SAA
Associated diseases	Plasma cell dyscrasias such as multiple myeloma, B cell lymphoma	Chronic inflammation, autoimmune diseases, cancers
Pathogenesis	Stimulus → monoclonal B cell-proliferation → excess of light chains → partial degradation → insoluble AL fibril	Stimulus → chronic inflammation → activation of macrophages → cytokines → partial degradation → insoluble AA
Incidence	More common in developed countries	Common worldwide
Distribution	Kidney, heart, bowel, nerves	Kidney, liver, spleen, adrenals
Stains	Specific immunoassays with anti-kappa antibodies	Immunoassays with anti-AA

Common Congenital Immune Deficiency Disease

1. **XLA (X-linked agammaglobulinaemia or Bruton disease)**
   (a) Failure of B cell maturation and absence of antibodies (due to mutations in BTK gene, which encodes B cell tyrosine kinase, required for delivering maturation signals from pre-B cells and B cell receptors)
   (b) Absence of gammaglobulin in the blood
   (c) Manifests by about 6 months of age, when there is depletion of maternal immunoglobulins
   (d) Patients are susceptible to recurrent bacterial or viral infections and infections with *Giardia lamblia*
2. **Common variable immunodeficiency**
   (a) Heterogeneous group of disorders characterized by hypogammaglobulinaemia, impaired immune response and increased susceptibility to infections
   (b) Onset in second decade
   (c) Defects in antibody production due to unknown cause
   (d) Plasma cells are absent, perhaps due to a block in antigen-stimulated B cell differentiation
   (e) These patients are also prone to develop autoimmune diseases as well as lymphoid tumours.
3. **Selective IgA deficiency:**
   (a) Most common of all primary immunodeficiencies.
   (b) Failure of IgA production due to unknown cause (seemingly caused by a block in the terminal differentiation of IgA-secreting B cells to plasma cells)
   (c) Since IgA is the most common immunoglobulin in mucosal surfaces, its deficiency leads to recurrent sinonasal and pulmonary infections as well as diarrhoea.
4. **X-linked SCID (severe combined immunodeficiency):** Failure of both T cell and B cell maturation due to the mutation in the common γ chain of the cytokine receptor, leading to failure of IL-7 signalling and defective haemopoiesis (IL-7 is the growth factor responsible for stimulating survival and expansion of B and T cell precursors).
5. **Autosomal SCID:** Failure of T cell development and a secondary defect in antibody responses, which are due to a defect in the gene coding for ADA (adenosine deaminase), leading to accumulation of toxic metabolites, which hamper lymphocyte maturation and proliferation. ADA is an enzyme involved in purine metabolism.
6. **X-linked hyper-IgM syndrome:**
   (a) In normal individuals, IgM is the first antibody to be produced by the body followed sequentially by IgG, IgA and IgE.
   (b) This orderly appearance of different antibody types is called *heavy chain class isotype switching*.
   (c) IgM-producing cells turn on the transcription of genes that encode for other isotypes, depending on the contact-mediated signals provided by the interaction between CD40 molecule on B cells and CD40L on activated T cells.
   (d) The most common genetic abnormality is mutation in the gene coding for CD40L (on X chromosome). Patients with this syndrome produce normal or even supernormal levels of IgM antibodies to antigens but lack the ability to produce IgG, IgA and IgE isotypes.
7. **Wiskott–Aldrich syndrome**
   (a) X-linked recessive disorder characterized by thrombocytopenia, eczema and a marked susceptibility to recurrent infections
   (b) Associated with a progressive age-related depletion of T lymphocytes in the peripheral blood and lymph nodes
   (c) Also, there is inability to synthesize antibodies to polysaccharide antigens and increased susceptibility to encapsulated pyogenic organisms.

Q. Write in detail on the etiopathogenesis of acquired immunodeficiency syndrome (AIDS).

Ans. AIDS is a disease caused by a retrovirus, human immunodeficiency virus (HIV). Two genetically different but related forms of HIV, namely HIV-1 and HIV-2, are implicated. Infection is characterized by depletion of CD4+ T cells (fewer than 200/μL in number).

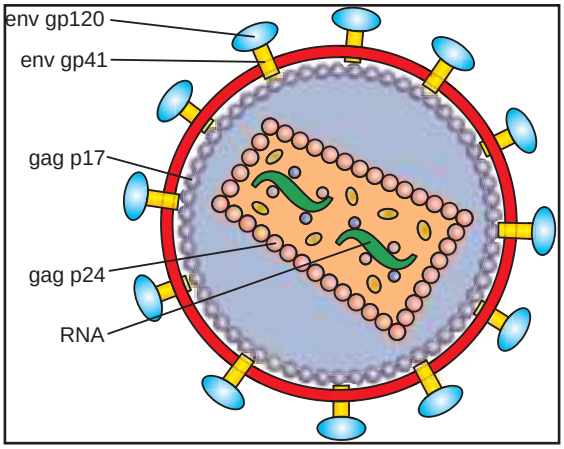


FIGURE 5.5. Structure of HIV virus.

Structure of HIV Virus (Fig. 5.5)

- HIV virus is spherical in shape and contains an electron-dense, cone-shaped core which further contains:
  - Major capsid protein p24
  - Nucleocapsid proteins p7/p9
  - Two copies of genomic RNA
  - Three viral enzymes (protease, reverse transcriptase and integrase)
- The viral core is surrounded by a matrix protein called p17.
- The viral envelope is studded with two glycoproteins, gp120 and gp 41, critical for infection.
- HIV proviral genome contains nonstructural and regulatory genes like *LTR*, *vif*, *vpr*, *vpu*, *nef* and *rev*, which code for different viral proteins (Table 5.15).

TABLE 5.15. HIV genes coding for different viral proteins

Gag gene	Capsid protein p24, matrix protein p17, nucleocapsid protein p7/9
Poll gene	Reverse transcriptase, protease, integrase, ribonuclease
Env gene	Envelope glycoprotein gp160, cleaved in endoplasmic reticulum to gp120 (mediates CD4 and chemokine receptor binding), and gp41 (mediates fusion)

Pathogenesis of HIV

Targets

1. **Immune system:** CD4+ T cells, macrophages/monocytes and dendritic cells/Langerhans cells

CD4+ T lymphocyte (Flowchart 5.14)

CD4+ molecule is a high-affinity receptor for HIV
↓
HIV gp120 binds with CD4 molecules
↓
Conformational changes in gp120 and formation of a new recognition site on it
↓
This new site binds to CCR5/CXCR4 (co-receptors) resulting in conformational change in gp41 with insertion of a fusion peptide present at tip of gp41 into target cell membrane
↓
Viral core containing genome enters cytoplasm of cell

FLOWCHART 5.14. Steps in the binding of HIV virus to CD4+ T lymphocyte.

Macrophages

- Act as a factory and reservoir for the virus and a vehicle for HIV to be transported to other organs
- Provide a site for viral replication in late phase of the disease, when CD4+ T cell count is greatly decreased
- *HIV may be macrophage tropic (R5 virus strains) or CD4+ T cell tropic*
- CCR5 (β-chemokine) receptors are present on monocytes/macrophage and freshly isolated peripheral blood T cells (not in vitro propagated T cell line)
- T cell tropic-CXCR4 (α-chemokine) receptors are present on T cells, both freshly isolated and culture retained
- M-tropic viruses are more efficient in transmitting AIDS but T-tropic HIV gradually accumulates and cause the final rapid phase of disease (being more virulent)
- On internalization, viral genome undergoes reverse transcription to form cDNA
- cDNA may remain in episomal form in quiescent T cells but may be integrated into host genome in dividing cells
- After integration, provirus may remain silent or may transcribe and form viral particles in activated T cell on exposure to antigen/cytokines *(clinical latency/chronic infection)*
- Release of viral particles results in CD4+ T cell lysis
- Qualitative defects in T cells are observed in T cells even in asymptomatic patients *(leading to clinical symptoms)*

Dendritic cells

- Mucosal Langerhans cells capture the virus and transport it to regional lymph nodes.
- Follicular dendritic cells in germinal centre of lymph nodes are reservoirs of HIV.

2. **CNS**
   - Viruses are carried to circulation by infected monocytes.
   - Viruses infect macrophages and microglia (HIV does not infect neurons).
   - Neurological deficit is due to direct effect of gp 120 or may be caused indirectly by viral products and soluble factors (IL-1, TNF-α, IL-6) produced by macrophages/microglia.

Q. Enumerate the abnormalities of immune functions in AIDS.

Ans. Abnormalities of immune functions:

1. **Lymphopenia**

Selective loss of CD4+ T helper-inducer cells with reversal of CD4:CD8 ratio

2. **Altered T cell functions in vitro**
   (a) ↓ Lymphocyte proliferative response to mitogens and antigens
   (b) ↓ Specific cytotoxicity
   (c) ↓ T helper cell function for B cells (decreased antibody production)
3. **Decreased T cell functions in vivo**
   (a) Loss of activated and memory T cells
   (b) Decreased type IV hypersensitivity
   (c) Susceptibility to opportunistic infections and neoplasms
4. **Altered monocyte/macrophage functions**
   (a) ↓ Chemotaxis and phagocytosis
   (b) ↓ HLA-II expression
   (c) ↓ antigen presentation
5. **Polyclonal B cell activation**
   (a) Hypergammaglobulinaemia and circulating immune complexes
   (b) Decreased ability to mount an antibody response to a new antigen
   (c) Loss of control/signals for B cell function in vitro

Q. Write briefly on Centers for Disease Control (CDC) classification of categories of HIV Infection.

Ans. The CDC classification of HIV infection is given in Table 5.16.

TABLE 5.16. CDC classification of HIV infection

Clinical categories	CD4+ T cell categories		
	1 (≥500 cells/μL)	2 (200–499 cells/μL)	3 (<200 cells/μL)
A. Asymptomatic, acute primary HIV, or persistent generalized lymphadenopathy	A1	A2	A3
B. Symptomatic, not A or C conditions	B1	B2	B3
C. AIDS indicator conditions: including constitutional disease, neurologic disease or neoplasm			

Note: Data from CDC, 1993, revised classification of AIDS.

Q. Enumerate the AIDS-defining opportunistic infections and neoplasms.

Ans. ***AIDS-defining opportunistic infections***:

Protozoal and Helminthic

- Cryptosporidiosis or isosporidiosis (enteritis)
- Toxoplasmosis (pneumonia or CNS infection)

Fungal

- Candidiasis (oesophageal, tracheal and pulmonary infections)
- Cryptococcosis (CNS infection)
- Coccidioidomycosis (disseminated infection)
- Histoplasmosis (disseminated infection)
- Pneumocystosis (pneumonia or disseminated infection)

Bacterial Infections

- Mycobacteriosis (*atypical* and *Mycobacterium tuberculosis*; pulmonary and extrapulmonary)
- Nocardiosis (pneumonia, meningitis and disseminated infections)
- *Salmonella* infection

Viral

- Cytomegalovirus (pulmonary, intestinal, retinal or CNS infections)
- Herpes simplex virus (localized or disseminated infection)
- Varicella zoster virus (localized or disseminated infection)
- Progressive multifocal leukoencephalopathy

AIDS-Defining Neoplasms

- Kaposi sarcoma
- B cell non-Hodgkin lymphoma
- Primary lymphoma of the brain
- Invasive cancer of the uterine cervix

Q. Write briefly on the laboratory diagnosis of AIDS.

Ans. Laboratory diagnosis of AIDS includes:

1. **Nonspecific tests**
   (a) Decreased TLC
   (b) Decreased lymphocyte count (<2000/mm^3)

(c) Decreased CD4+ T cell count (<200 CD4+ T cells/μL) and reversal of T4:T8 ratio
(d) Thrombocytopenia
(e) Increased β2 microglobulin level
(f) Lymph node biopsy:
  (i) Early stage
    - Marked follicular hyperplasia
    - Follicles extend to medulla and sometimes spread outside the capsule
    - Mantle zone thinned out and germinal centres seem to merge with the interfollicular areas
    - Presence of monocytoid B cells in and around sinusoids and in trabecular blood vessels
    - Involvement of the B cell areas of the lymph node supports polyclonal B cell activation and hypergammaglobulinaemia
  (ii) Disease progression
    - Severe follicular involution
    - Follicles are depleted of cells
    - Organized network of follicular dendritic cells disrupted
    - Germinal centres become hyalinized
    - Atrophic and small lymph nodes *(burnt out appearance)*
    - In this stage, lymph nodes may harbour opportunistic infections
    - The inflammatory response to infections in both nodal and extranodal sites may be atypical and sparse, eg, granulomatous response to mycobacteria may not develop adequately because of deficient CD4+ T cells.

2. **Specific tests**
(a) Antigen detection
  (i) Acute illness/seroconversion stage: p24 antigenaemia and viraemia, also appearance of IgM thereafter
  (ii) Asymptomatic phase: decreased or absent free p24, but antibody-bound p24 antigen may be demonstrated
  (iii) Clinical disease: increased free p24 antigen

Method: Antigen-capture ELISA

In the first few weeks after infection and in terminal phase, the test is uniformly positive.

(b) Antibody detection
  (i) Simplest and most widely used method
  (ii) Negative in *window period* that follows infection (time taken for antibodies to appear); IgM appears first followed by IgG
  (iii) ELISA:
    - Sensitive but not so specific
    - *Types used*: 'Direct solid phase antiglobulin ELISA' and 'Capture ELISA specific for IgM antibody'
  (iv) Western blot test: more specific than ELISA
  (v) PCR: Now 'new gold standard' test for diagnosis in all stages of HIV
(c) Direct virus isolation and culture in neoplastic T cell line

6

Neoplasia

Q. Define neoplasia.

Ans. Neoplasia (new growth) is excessive and unregulated proliferation that eventually becomes autonomous (independent of physiologic growth stimuli).

Q. Define oncology.

Ans. Oncology is the study of the tumours or neoplasms (oncos in Greek means tumour).

Q. Define cancer.

Ans. Cancer is the common term for malignant tumours (derived from the Latin word crab, indicating adherence to any part it seizes upon obstinately like a crab).

Q. What is clonality?

Ans. A tumour is said to be clonal when the entire population of cells within a tumour arises from a single cell that has incurred genetic change. A clonal neoplasm is therefore constituted by cells which carry the same genetic anomaly, eg, in lymphoma and leukaemia, clonality is proven by the amplification of a single rearrangement of their immunoglobulin gene (for B cell lesions) or T cell receptor gene (for T cell lesions). The demonstration of clonality is now considered to be necessary to identify a lymphoid cell proliferation as neoplastic; however, as this is not always possible, clonality is not included in the definition of neoplasia.

Q. What are the two main components of all neoplasms?

Ans. Histologically, almost all neoplasms are composed of two main components:

1) **Tumour cells that comprise the parenchyma** (also called specific component).
2) **Tumour stroma** which is a supporting framework consisting of connective tissue and newly formed blood vessels elicited from adjacent tissues.

There is perpetual interaction between parenchyma and stroma, which directly influences the growth of the tumour.

Q. What is desmoplasia?

Ans. Hyperplasia of fibroblasts and formation of abundant collagen in the stroma as a reaction to infiltration by a cancer is labelled desmoplasia.

Q. Define a teratoma.

Ans. Teratoma is a tumour derived from a variety of cell types representing more than one germ cell layer, usually all three.

Characteristics:

- Derived from totipotent cells (cells with an ability to differentiate into any cell type), teratomas are usually encountered in gonads. They sometimes develop in sequestered primitive cell rests elsewhere.
- Sacrococcygeal teratomas are the most common tumours in newborns, and mature cystic teratomas account for 10–20% of all ovarian neoplasms.
- Teratomas are also frequently seen in the head and neck region, mediastinum and retroperitoneum.

Q. Define a choristoma.

Ans. A choristoma is an ectopic rest of normal tissue, eg, a rest of adrenal cells under the kidney capsule and pancreatic rest in the intestine.

Q. Define a hamartoma.

Ans. Aberrant differentiation may produce a mass of disorganized but mature, specialized cells/tissue indigenous to the particular site (thought to be either an anomalous development or a neoplasm in origin), eg, hamartoma of the lung.

Q. Differentiate between a hamartoma and a neoplasm.

Ans. Differences between a hamartoma and a neoplasm are summarized in Table 6.1.

TABLE 6.1. Differences between a hamartoma and a neoplasm

Features	Hamartoma	Neoplasm
Definition	Disorganized focal overgrowth of mature tissue indigenous to a particular site	Abnormal, excessive, unregulated, autonomous proliferation of cells
Behaviour	Always benign	May be benign or malignant
Degree of differentiation	Well-differentiated cells, which completely resemble normal counterparts	Vary from well-differentiated to poorly differentiated anaplastic lesions
Clonality	Polyclonal	Monoclonal
Examples	Vascular hamartoma	Squamous cell carcinoma

Classification of Neoplasms

Neoplasms can be classified into different types based on the following features:

1. **Gross or naked-eye appearance:**
   - **Benign lesions** are usually encapsulated and circumscribed and grow along broad fonds (have pushing margins).
   - **Malignant lesions** are usually unencapsulated, ill-defined and infiltrating. They can have several different gross appearances, that is, annular (endophytic), ulcerative, fungating (exophytic or cauliflower-like), scirrhous (showing excessive fibrosis) or mucoid (containing abundant mucin).
2. **Histological appearance and histogenetic/embryological considerations:**
   - **Cell of origin**, ie, epithelial or connective tissue, undifferentiated stem cells or highly specialized cells/tissue.
   - **Vascular/lymphatic invasion**
   - **Capsular invasion**
   - **Histopathological margins** (infiltrating or expansile)
3. **Behavioural characteristics** (indolent, borderline aggressive or frankly aggressive)
4. **Aetiological characteristics** (tumour induced by radiation, chemical or viral carcinogens; tumour of primary or secondary origin)
5. **Functional characteristics** (some tumours secrete hormones or proteins with characteristic effects on the body, eg, adrenocorticotropic hormone (ACTH), parathormone (PTH) and antidiuretic hormone (ADH) secreted by lung carcinoma and keratin produced by well-differentiated squamous cell carcinoma)

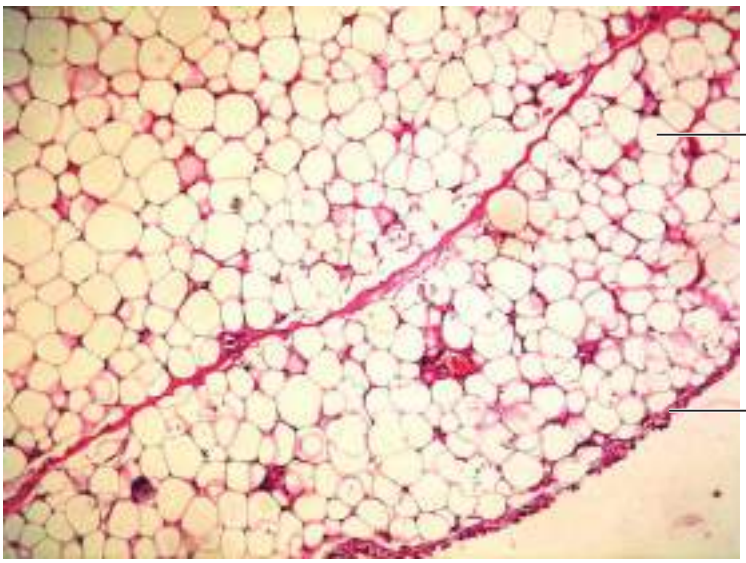


FIGURE 6.1. Lipoma composed of mature adipocytes and surrounded by a well-formed capsule indicating its benign nature (H&E; 100×).

Presently, tissue of origin and behavioural pattern is the basis of classification of most neoplasms.

Q. Define and classify benign tumours?

Ans. Benign tumours are neoplasms, which grow as cohesive expansile masses, which do not invade, **infiltrate** or **metastasize.** They are usually encapsulated. (The capsule is made of a rim of compressed connective tissue derived largely from the native stroma.)

Nomenclature and Classification

1. **Tumours of mesenchymal origin:** Designated by adding suffix 'oma' to the cell of origin, eg, fibroma, lipoma (Fig. 6.1), osteoma and chondroma.
2. **Tumours of epithelial origin are variously classified:**
   (a) Some based on the cell of origin, eg, squamous cell carcinoma.
   (b) Others based on the microscopic architecture, eg, adenoma (glandular pattern), papilloma (finger-like or warty projections), cystadenoma, (cystic masses) and papillary cyst adenoma (papillary cystic masses)
3. **Mixed tumours:** Divergent differentiation of a single line of parenchymal cells resulting in tumours comprised more than one cell type; usually derived from one germ cell layer, eg, pleomorphic adenoma of the salivary gland.

Q. Define differentiation?

Ans. Differentiation is the extent to which neoplastic cells resemble comparable normal cells, both morphologically and functionally.

- The cells in benign tumours are almost always well differentiated and resemble their normal cells of origin. Cancers, however, vary from being well differentiated to poorly differentiated.
- **Well-differentiated cancers** show progressive maturation or specialization of undifferentiated cells as they proliferate. **Poorly differentiated or undifferentiated cancers** show proliferation without differentiation or maturation.
- Well-differentiated squamous cell carcinomas of the epidermis elaborate keratin, just as well-differentiated hepatocellular carcinomas elaborate bile. Highly anaplastic undifferentiated cells, whatever is their tissue of origin, loose their resemblance to the normal cells from which they have arisen.

Q. Define anaplasia. Enumerate the morphological features indicative of anaplasia.

Ans. Anaplasia is defined as lack of differentiation; literal meaning is 'reverse differentiation'. It is a hallmark of malignant transformation.

Morphological Features Indicative of Anaplasia

- Pleomorphism (variation in size and shape of cells)
- Anisonucleosis (variation in size of nuclei)
- Abnormal nuclear morphology:
  - Abundant and darkly staining (hyperchromatic) DNA
  - Coarsely clumped chromatin or clumping of nuclear chromatin along the nuclear membrane resulting in prominent appearing nucleoli
  - Increased nucleocytoplasmic ratio (normal from 1:4 to 1:6; may approach 1:1)
- Numerous mitoses with abnormal, atypical, bizarre, tri, quadri and multipolar spindles (abnormal mitoses are seen in malignant tumours only)
- Loss of polarity (orientation of cells)
- Presence of the tumour giant cells

Q. Define dysplasia. Enumerate the steps in the course of progression of dysplasia to invasive cancer.

Ans. Dysplasia (disordered growth) is defined as the loss of architectural orientation of cells with respect to one another and presence of pleomorphism, nuclear hyperchromatism and mitoses. Dysplasia may sometimes (not always) progress to invasive carcinoma (Flowchart 6.1; Fig. 6.2).

Carcinoma in situ ('cancer in place') is a lesion in which the dysplastic cells show essentially no maturation and grow rapidly without regulation; however, they remain localized, and do not invade past the basement membrane into the subepithelial tissue or stroma.

Invasive carcinoma is the final step in this sequence of events. It is the stage in which the malignant cells have invaded beyond the basement membrane and have acquired the potential to metastasize. Invasive carcinoma, if left untreated, is almost always fatal.

Dysplasia

↓

Carcinoma in situ

(Dysplasia involving the entire thickness of the epithelium)

↓

Invasive carcinoma

(Frank invasion into the stroma by malignant cells)

FLOWCHART 6.1. Steps in the progression of dysplasia to invasive carcinoma.

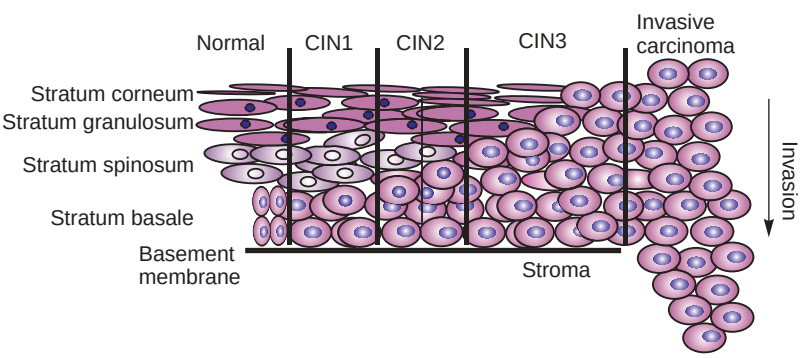


FIGURE 6.2. Sequential progression of dysplasia to invasive carcinoma.

Q. Differentiate between dysplasia and anaplasia.

Ans. Differences between dysplasia and anaplasia are shown in Table 6.2.

TABLE 6.2. Differences between dysplasia and anaplasia

Features	Dysplasia	Anaplasia
Definition	Lack of uniformity of individual cells with architectural distortion	Lack of morphological and functional differentiation of cells
Behaviour	A potentially precancerous condition, which may or may not progress to cancer	Anaplasia is usually a hallmark of malignant transformation
Tissue involved	Mainly epithelium	Both epithelium and mesenchyme
Cellular pleomorphism and nuclear atypia	Present, but usually low grade	High grade
Mitotic figures	Present, usually not atypical	Abnormal and atypical figures may be seen (tripolar, quadripolar and multipolar spindles)
Tumour giant cells	Absent	Present

Q. Differentiate between metaplasia and dysplasia.

Ans. Differences between metaplasia and dysplasia are enlisted in Table 6.3.

TABLE 6.3. Differences between metaplasia and dysplasia

Features	Metaplasia	Dysplasia
Definition	Reversible change in which one adult cell type (epithelial or mesenchymal) is replaced by another adult cell type	Loss of uniformity of the individual cells (mainly epithelial) as well as lack of architectural orientation with respect to one another
Cellular pleomorphism	Usually not seen	Present
Nuclear atypia	Usually not seen	Hyperchromatic and abnormally large atypical nuclei may be seen
Mitotic figures	Few	Many
Orientation with respect to one another (tissue architecture)	Maintained	Loss of ordered maturation as in dysplastic stratified squamous epithelium
Reversibility	Reversible, if triggering factors are removed	May become irreversible if it involves the whole thickness of the epithelium
Example	Columnar to squamous epithelium in respiratory tract of chronic smokers	Cervical intraepithelial neoplasia (CIN)

Q. Define and classify malignant tumours.

Ans. Malignant tumours are neoplasms which **infiltrate**, **invade** and **metastasize.** They may be

- **Mesenchymal** or **connective tissue in origin,** eg, sarcomas
- **Epithelial in origin,** eg, carcinomas, usually named after their parent organ or tissue of origin, eg, adenocarcinoma of intestine and squamous cell carcinoma of the cervix or oral mucosa (Fig. 6.3)
- **Poorly differentiated** or **undifferentiated malignant tumours,** eg, cancers composed of undifferentiated cells or cells of unknown origin

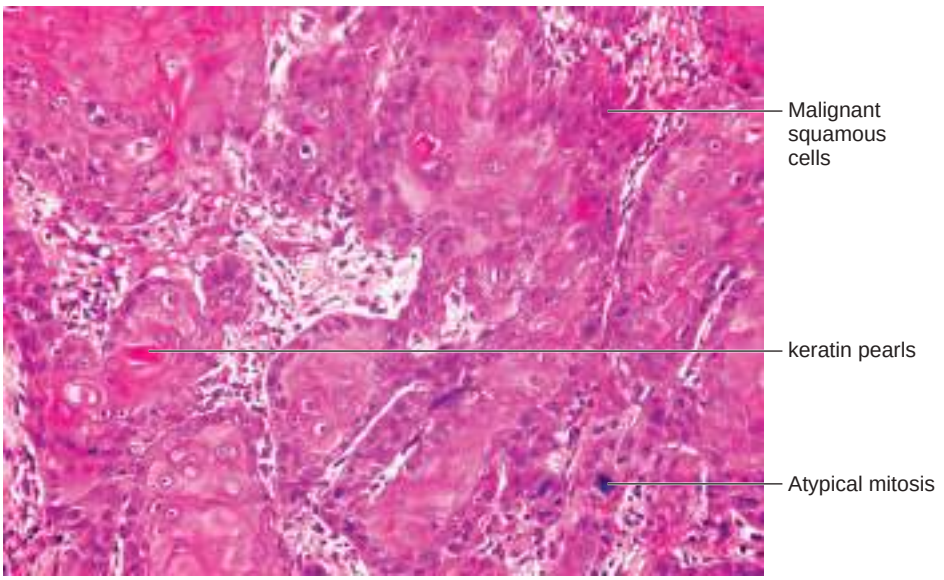


FIGURE 6.3. Well-differentiated squamous cell carcinoma of oral mucosa comprising anaplastic squamous cells, at places, forming keratin pearls (H&E; 200×).

Q. Differentiate between benign and malignant tumours.

Ans. Differences between benign and malignant tumours are shown in Table 6.4.

TABLE 6.4. Contrasting features of benign and malignant tumours

Features	Benign	Malignant
Gross features		
Boundaries	Encapsulated/well circumscribed	Ill circumscribed/unencapsulated
Size	Usually small	Usually large
Secondary changes	Less frequent	More frequent
Surrounding tissue	Compressed	Invaded
Microscopic features		
Pattern	Resembles tissue of origin	Poor resemblance to tissue of origin
Polarity	Retained	Lost
Anaplasia	Absent	Present
Mitoses	Present, few, typical	Present, many, atypical as well as typical
Tumour giant cells	Rare, without atypia	Common, with atypia
Cytogenetic changes	Rare	Common
Physiology of cells/function	Maintained	Lost
Growth rate	Low	High
Local invasion	Rare	Common
Metastasis	Absent	Present

Q. Define local invasion.

Ans. Most cancers are accompanied by progressive infiltration and destruction of the surrounding tissue, referred to as local invasion.

Q. Define metastasis. Write briefly on the various pathways of spread of the tumours.

Ans. Tumour implants discontinuous with the primary tumour, confirm the malignant nature of a tumour and are labelled metastases. All cancers metastasize with a few exceptions, eg, basal cell carcinoma (rodent ulcer) and gliomas of the central nervous system, which are locally invasive and rarely metastasize.

Pathways of Spread of the Tumours

1. **Direct seeding of body cavities and surfaces:** Penetration of a tumour into a natural open field/space, eg, pleural, pericardial, subarachnoid and synovial. Sometimes mucinous tumours of appendix and ovary (both benign and malignant) fill the peritoneal cavity with a gelatinous neoplastic mass called **'pseudomyxoma peritonei'.**
2. **Lymphatic spread**
   (a) There are numerous interconnections between lymphatic and vascular channels; so, emphasis on differentiating lymphatic and vascular dissemination may be purposeless.
   (b) Functional lymphatics are absent in tumours and lymphatic vessels located at the surface are sufficient for lymphatic spread.
   (c) Lymphatic spread tends to follow natural routes of lymphatic drainage and is the usual route for dissemination of **epithelial malignancies (**Fig. 6.4**)**; sarcomas may also use this route.
   (d) Drainage of tumour cell debris and antigens may induce reactive hyperplasia and the spread of tumour cells to regional lymph nodes.
   (e) A '**sentinel' lymph node** is defined as the first node in the regional lymphatic chain to receive lymph flow from the primary tumour.
3. **Haematogenous spread**
   (a) Typical of **sarcomas** but also seen in carcinomas
   (b) Arteries have thick walls, are less penetrable than veins
   (c) All portal blood flows to liver and all caval blood flows to lungs; therefore, **liver and lungs are the most frequently involved organs in haematogenous dissemination**
   (d) Cancers in the vicinity of vertebral column, eg, thyroid and prostate, metastasize to the vertebrae via paravertebral plexus

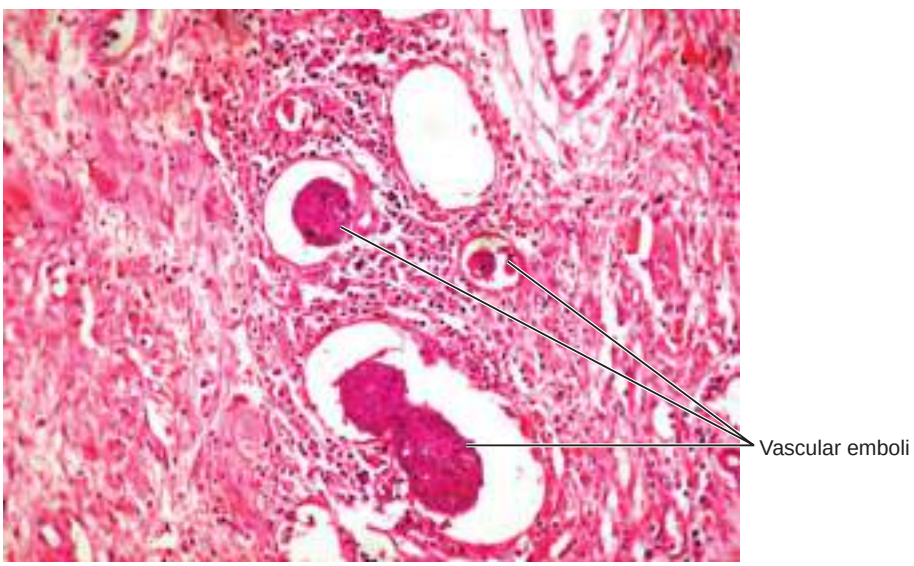


FIGURE 6.4. Section showing vascular tumour emboli (H&E; 200×).

Q. Write briefly about the biology of the tumour growth.

Ans. Rate of growth of the tumour depends on:

- **Doubling time** of tumour cells (time taken by the tumour cells to double their volume or number of cells).
- **Growth fraction** (fraction of the tumour cells that are in the replicative pool).
- Rate at which cells are shed and lost in the growing lesion.
- **Fast-growing tumours have a high cell turnover** (high rate of, both proliferation and apoptosis); the tumour growth is possible only if rate of proliferation is greater than rate of apoptosis.
- Most anticancer drugs act on cells, which are in the growth cycle.
- Tumours with high growth fraction are more susceptible to radio and chemotherapy.
- Debulking with surgery and radiation shifts tumour cells from G_0 to G_1 phase.
- Rate of growth is proportional to the level of differentiation of cells.

Q. Write briefly about the epidemiology of malignant tumours.

Ans. Cancer is a leading cause of death worldwide, accounting for a large number of deaths. Lung, stomach, liver, colon and breast cancer cause most cancer deaths each year. The frequency of different cancers differs between men and women. Most cancers can be attributed to five leading behavioural and dietary risks: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco and alcohol use. The following are the commonest malignant tumours encountered worldwide:

Men:
Prostate (oral cavity in India)
Lungs
Colon or rectum
Leukaemia and lymphoma
Liver

Women:
Breast (cervix in India)
Lungs
Colon or rectum
Leukaemia and lymphoma
Ovary

Children:
Acute leukaemia
CNS tumours
Lymphomas
Neuroblastoma
Bone sarcomas

Epidemiological Factors Influencing Neoplasia

Familial and Genetic Factors (Inherited Predisposition to Cancer)

1. **Autosomal dominant inherited cancer syndromes**
   The autosomal dominant inheritance of a single mutant gene greatly increases risk of developing a tumour. The inherited mutation is generally a point mutation occurring in a single allele of a tumour suppressor gene. Defect in the second allele occurs in the somatic cell, as a consequence of deletion or recombination. As in other autosomal dominant conditions, both incomplete penetrance and variable expressivity occur. *Examples are as follows:*
   (a) Retinoblastoma (RB): Approximately 40% of retinoblastomas (RBs) are inherited. Carriers of a mutant RB tumour suppressor gene have a 10,000-fold increased risk of developing RB, usually bilateral. They also have a greatly increased risk of developing a second cancer, usually osteogenic sarcoma.

(b) Familial polyposis coli (FAP):
- Presents with polypoid adenomas at birth and shows 100% conversion to familial polyposis coli by age of 50 years.
- The genetic defect in FAP is a germline mutation in the adenomatous polyposis coli (APC) gene. Syndromes once thought to be distinct from FAP are now recognized to be a part of the phenotypic spectrum of FAP.
- Syndromes with a germline mutation in the APC gene include FAP, Gardner syndrome, some families with Turcot syndrome and attenuated adenomatous polyposis coli (AAPC).
- **Gardner syndrome** is characterized by colonic polyposis typical of FAP, along with osteomas (skull and mandible), dental abnormalities and soft tissue tumours. **Turcot syndrome** is characterized by colonic polyposis typical of FAP, along with CNS tumours (medulloblastoma). **AAPC** is characterized by fewer colonic polyps as compared to classic FAP, which tend to develop late (average age 36 years), and involve proximal colonic area.

(c) Multiple endocrine neoplasia (MEN) syndromes:
- The term **MEN** encompasses several distinct autosomal dominant syndromes featuring both benign and malignant tumours derived from endocrine and nonendocrine tissue.
- There are two major forms of MEN—type 1 and type 2—which are distinguished based on the genes involved and hormones secreted (**MEN 1—** adenomas of pituitary, parathyroid and pancreas; **MEN 2—**medullary carcinoma thyroid, pheochromocytoma and parathyroid tumours).
- Other component neoplasms include adrenocortical tumours; visceral and cutaneous lipomas; meningiomas; facial angiofibromas and thymic, gastric and bronchial carcinoids.
- MEN 1 follows Knudson's 'two-hit' model for tumour suppressor gene carcinogenesis. First hit is a heterozygous MEN 1 germline mutation, inherited from one parent (familial cases) or developed in an early embryonic stage (sporadic cases) and present in all cells at birth. Second hit is a MEN 1 somatic mutation, usually a large deletion, which occurs in predisposed endocrine cells. MEN 2 is caused by a mutation of RET proto-oncogene.

(d) Neurofibromatosis (NF) or von Recklinghausen disease:
- NF is an autosomal dominant disorder that affects bones, nervous system, soft tissue and skin.
- At least eight different clinical phenotypes of NF have been identified, and they are linked to at least two genetic disorders. Commonly abbreviated **NF1** (neurofibromatosis type 1), the first is also known as **von Recklinghausen disease.** It occurs following mutation of neurofibromin 1 on chromosome 17q11.2.
- Neurofibromin is a tumour suppressor gene whose function is to inhibit p21 RAS oncoprotein. In absence of the inhibitory control of RAS oncoprotein by this gene, there is uncontrolled cellular proliferation and manifestation mainly as neurofibromas and Café au lait spots.
- **Neurofibromatosis type 2** (also called **'central neurofibromatosis'**) is a result of mutation of protein merlin (also known as 'Neurofibromin 2' or 'schwannomin') on chromosome 22q12. It accounts for only 10% of all cases of NF. Normal function of merlin is not well understood.

(e) Li-Fraumeni syndrome: Results from germline mutation of P53 (see P53).

2. **Defective DNA repair syndromes**

Human syndromes with DNA repair deficiencies cause genomic instability which is the driving force behind cancer development. There are four categories of DNA repair genes:
(a) Mismatch repair genes
(b) Base excision repair genes
(c) Nucleotide excision repair genes
(d) Double strand break repair genes

Mostly autosomal recessive in inheritance, common examples of DNA repair defects include **xeroderma pigmentosa, ataxia telangiectasia** and **Bloom syndrome**. Xeroderma pigmentosa manifests with extreme sensitivity to UV

radiation and predisposition to basal cell carcinoma, squamous cell carcinoma and malignant melanoma. Rarely defective DNA repair syndromes may be autosomal dominant in inheritance, as in HNPCC (hereditary nonpolypoid colonic cancer caused by inactivation of a DNA mismatch repair gene).

3. **Familial cancers**

Cancers with a high frequency of occurrence within a family are called familial cancers, eg, carcinomas of breast, colon, uterus, ovary, stomach and some sarcomas. Familial cancers have the following features:

(a) Early age of onset
(b) Multiple primary cancers in a single individual (such as bilateral breast cancer)
(c) No clearly identifiable pattern of transmission

Racial and Geographic Factors (Table 6.5)

TABLE 6.5. Association of racial and geographic factors with cancers

Race	Commonly seen cancers
White Europeans	Carcinoma skin, penis, cervix and liver
Japanese	Carcinoma stomach
Indians	Oral and GIT cancers, carcinoma cervix and breast

Environmental and Cultural Factors

1. **Cigarette smoking** is known to be associated with oral cancer, carcinoma of larynx, pharynx, oesophagus, lungs, pancreas and urinary bladder.
2. **Alcohol abuse** causes cancers of oropharynx, larynx, oesophagus and liver. Alcohol and smoking together increase the incidence of cancer of the upper airways and digestive tract.
3. **Industrial and environmental carcinogens** include UV rays, smog, arsenic, asbestos, benzene, vinyl chloride and β-naphthylamine.
4. **Diet:** Following factors predispose to malignancies:
   (a) Overweight individuals
   (b) Deficiency of vitamin A, tocopherols, selenium and zinc
   (c) Diet rich in animal fats; low in fibre content
5. **Age:** Most cancers are seen after fifth decade; some cancers may be seen in childhood.
6. **Sex:** Males are more commonly affected, except in carcinoma breast, gall bladder, thyroid and hypopharynx.

Predisposing factors for specific malignancies:

- **Carcinoma of cervix:** Young age at first coitus, high frequency of sexual intercourse, multiplicity of partners and multiparity contribute to increasing probability of carcinoma cervix.
- **Penile carcinoma:** Rare in Jews and Muslims (because they are customarily circumcised); common in other communities.
- **Cancer of cheek and tongue:** Associated with betel nut and tobacco chewing.

Interactions between Genetic and Nongenetic Factors

Inherited variations (polymorphisms) in enzymes that metabolize procarcinogens to carcinogens can determine the susceptibility to cancer, eg, polymorphisms in gene coding for *P-450* confers inherited susceptibility to lung cancer in smokers.

Nonhereditary Predisposing Conditions

- **Chronic inflammation and cancer:**
  - An increased risk of cancer has been noted in chronic inflammatory conditions, eg, ulcerative colitis, *Helicobacter pylori* gastritis and viral hepatitis.

  - Inflammation increases expression of cyclooxygenase-2 (COX-2), which induces conversion of arachidonic acid into prostaglandins, which in turn are found to be increased in cancers, eg, colonic cancer. The role of COX-2 inhibitors in cancer treatment and the potential association between cancer and chronic inflammation is being explored.
  - Chronic inflammation is associated with repair and proliferation, thus increasing the tissue stem cell pool, which is vulnerable and susceptible to transformation,
- **Precancerous conditions:** Certain non-neoplastic and benign disorders have a well-defined association with cancer. Increased incidence of cancer in precancerous lesions is mostly attributed to continuous regenerative cell replication. These lesions include:
  - Actinic or solar keratosis
  - Barrett oesophagus
  - Leukoplakia of oral cavity, vulva and penis
  - Chronic atrophic gastritis
  - Paget disease of bone
  - Multiple villous adenomas of colon
  - Neurofibromatosis
  - Long-standing ulcerative colitis
  - Cirrhosis of liver
  - Chronic bronchitis (heavy smokers)
  - Chronic irritation of the oral cavity
  - Old burn scar (Marjolin ulcer)
- **Immunodeficiency states:** Individuals with deficient T cell immunity are predisposed to cancers particularly those caused by oncogenic viruses.

Q. Write in detail on the molecular basis of cancer.

Ans. Carcinogenesis is initiated by nonlethal genetic damage to a cell (**mutation**), which could be: (*a*) inherited in germ line or (*b*) acquired (due to chemicals, radiation and viruses). Occurrence of a mutation is followed by clonal expansion of the mutated cell. Mutations that result in development of malignancy are called **'driver mutations'**. The first driver mutation is called an **'initiating mutation'.** In addition to frank mutations, there are **'epigenetic aberrations'** which also contribute to malignancy, eg, DNA methylation and histone modifications. Unlike mutations, epigenetic aberrations are potentially reversible with drugs that reduce the influence of DNA or histone modifying factors leading to increasing interest in them. There are four main classes of regulatory genes:

1. Proto-oncogenes
   - Dominant
   - Cause 'gain of function' (excessively increase one or more of the normal functions of the encoded gene product)
   - Can transform cells despite the presence of a normal counterpart
2. Tumour suppressor genes
   - Recessive
   - Mutations in these genes cause a 'loss of function'
   - Both normal alleles of tumour suppressor genes must be damaged to transform cell
3. Genes regulating apoptosis and cancer
   - Apoptosis in a normal cell is guided by cell death receptor CD95.
   - *BAD*, *BID*, *BAX* and TP58 are proapoptotic.
   - *Bcl*-2 and *Bcl*-X are antiapoptotic.
   - Mutant form of *Bcl*-2 gene is seen in B cell lymphomas, carcinoma breast, thyroid and prostate.
   - CD95 is depleted in hepatocellular carcinoma.
4. DNA repair genes
   - Defects in DNA repair genes predispose to mutations (mutator phenotype).
   - Both alleles of DNA repair genes must be inactivated to induce genomic instability (recessive inheritance).

Q. Differentiate between anti-oncogenes and proto-oncogenes.

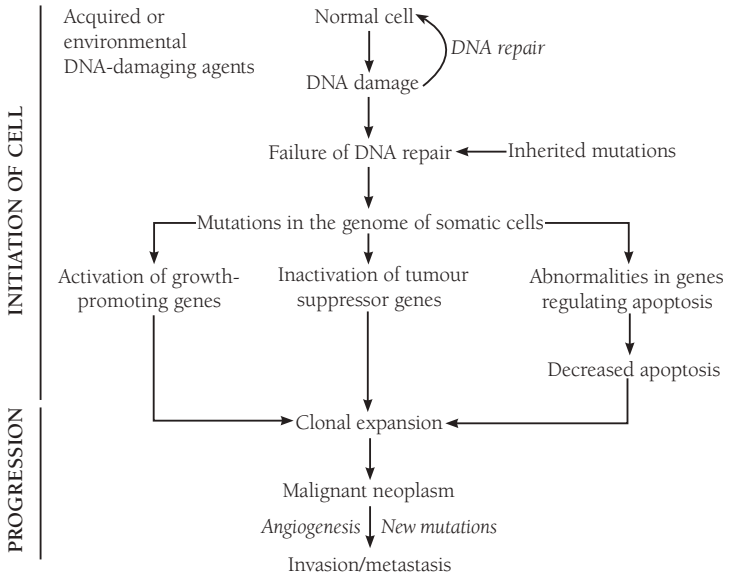
Ans. Differences between anti-oncogenes and proto-oncogenes are enlisted in Table 6.6.

TABLE 6.6. Differences between anti-oncogenes and proto-oncogenes

Features	Anti-oncogenes	Proto-oncogenes
Other name	Tumour suppressor genes	Precursor genes for oncogene
Function	They suppress cell proliferation, and promote differentiation and maturation of cells	They promote normal cell growth and differentiation
Inheritance	Recessive. Homozygous inactivation, ie, loss of both normal copies of gene is required for carcinogenesis	Dominant. Mutation in a single copy may lead to oncogenic conversion
Action	Act passively, ie, cancer-promoting genes dominate and lead to tumour formation due to loss of normal function of anti-oncogenes	Act actively, ie, gene products of oncogenes directly lead to tumour formation
Examples	*P53*, *RB* gene, *BRCA*-1 and 2, *TGF*-β, *APC*, *WT-1* and *NF*	*myc*, *N-myc*, *erb*B1/2/3

Q. Enumerate the steps involved in carcinogenesis.

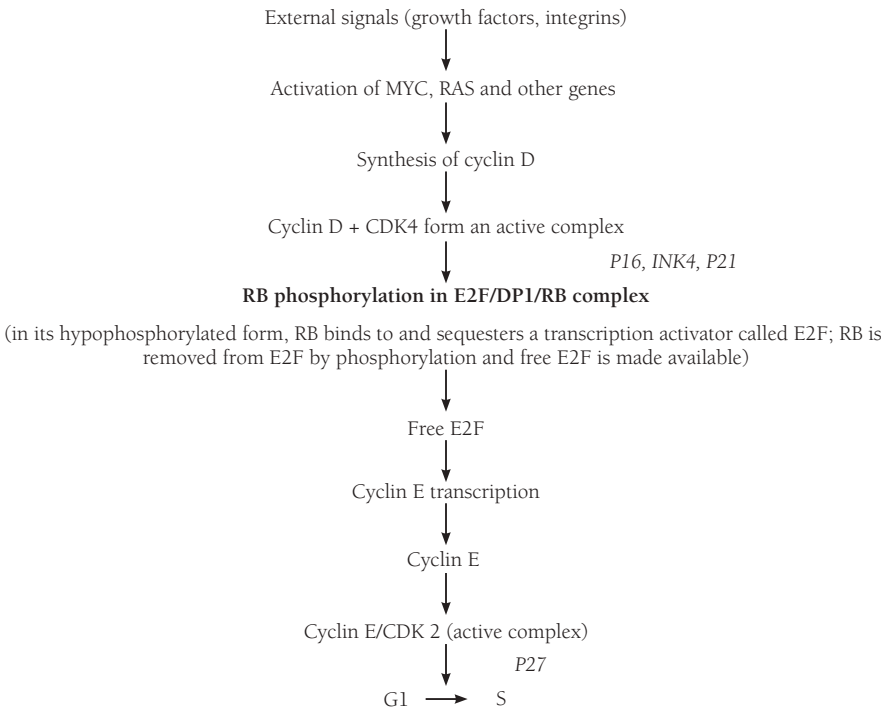
Ans. Carcinogenesis is a multistep process (Flowchart 6.2).



FLOWCHART 6.2. Steps involved in carcinogenesis.

Q. Write briefly on role of cyclins, CDK (cyclin-dependent kinases) and CDK inhibitors in regulating G_1/S cell cycle transition.

Ans. Cyclin D, the first cyclin to increase in the cell cycle, appears in mid-G phase, but is no longer detectable in the S phase. There are three forms of cyclin D, named Dl, D2 and D3; but to simplify matters, the general term 'cyclin D' is used. Cyclin D, like other cyclins, is unstable and is degraded through **ubiquitin—proteasome pathway**. During the G phase of the cell cycle, cyclin D binds to and activates CDK4, forming a *cyclin D–CDK4 complex*. This complex plays a critical role in the cell cycle by phosphorylating the retinoblastoma susceptibility protein (pRB). '**The phosphorylation of RB is a molecular ON–OFF switch for the cell cycle**.' In its hypophosphorylated state, RB prevents cells from replicating by forming a tight, inactive complex with the transcription factor E2F. Phosphorylation of RB dissociates the complex and releases the inhibition on E2F transcriptional activity (see Flowchart 6.3).



Note: The cell cycle is blocked by P21, P27, P16 and INK4.

FLOWCHART 6.3. Role of cyclins, CDKs and CDK inhibitors in regulating G1/S cell cycle transition.

Q. Write in detail on cancer-related genes and their effect on cell growth.

Ans. Alterations in the controlling genes, typically by mutation, are major genetic hallmarks of cancer and include:

Excessive and Autonomous Growth

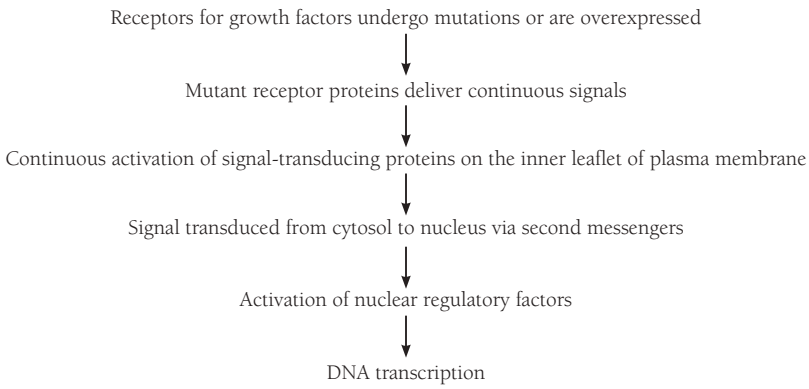
- Genes that promote autonomous cell growth in cancer cells are called **oncogenes** and their normal cellular counterparts are called **proto-oncogenes.**
- Proto-oncogenes are physiological regulators of cell proliferation and differentiation, and are often involved in signal transduction and execution of mitogenic signals, usually through their protein products.
- Oncogenes are mutated forms of normal proto-oncogenes, different from the latter in the following ways:
  (a) Presence of mutation/s in the structure of the gene
  (b) Ability to promote autonomous and excessive cellular proliferation in the event of overexpression, even in the absence of normal mitogenic signals
- Activation of oncogenes can be induced by
  (a) *Point mutation* and *deletion* (as in *RAS* oncogene implicated in carcinoma of colon and pancreas)
  (b) *Chromosomal translocation*, as in Philadelphia chromosome (translocation of C-ABL proto-oncogene from chromosome 9 to chromosome 22) and translocation of *c-MYC* proto-oncogene from chromosome 8 to chromosome 14 (seen in 75% cases of Burkitt lymphoma)
  (c) *Gene amplification* (chromosomal alterations that result in increased number of copies of a gene) as in the case of *n-MYC* implicated in neuroblastoma, and ERB-B2 in breast and ovarian cancer. The expression of oncogenes can be regulated by **microRNAs**

(miRNAs)—small RNAs 21–25 nucleotides in length that control gene expression by downregulating them. Mutations in such miRNAs (known as *oncomirs*) can lead to the activation of oncogenes.

- Oncoproteins are products of oncogenes, which resemble products of proto-oncogenes, but are devoid of important regulatory mechanisms. They include:

(a) *Growth factors (GFs)*:
- These are polypeptides elaborated by many cells that normally act on another cell to stimulate its proliferation (paracrine action), eg, PDGF in glioblastomas, TGF-α in sarcomas and FGF in carcinoma of bowel and breast.
- Many cancer cells are capable of synthesizing the same growth factors that stimulate their growth. An oncogene may cause a cell to secrete growth factors, even though it does not normally do so, thus inducing its own uncontrolled proliferation (*autocrine loop*) and proliferation of neighbouring cells, eg, osteosarcomas encode β-chain of PDGF and the same tumours also express receptors for PDGF.
- A group of related oncogenes that encode homologues of fibroblast growth factors (FGFs), eg, *HSTF*-1 (heparin-binding secretory transforming factor-1) and *INT*-2 (also known as fibroblast growth factor-3) are activated in several gastrointestinal and breast tumours; β *FGF*, a member of the fibroblast growth factor family, is expressed in human melanomas but not in normal melanocytes. Hepatocyte growth factor and its receptor *c-MET* are overexpressed in follicular carcinoma of thyroid.

(b) *Growth factor receptors*: Receptor kinases add phosphate groups to receptor proteins at the surface of the cell (which receive protein signals from outside the cell and transmit them to the inside of the cell). Tyrosine kinases add phosphate groups to the amino acid tyrosine in the target protein. They can cause cancer by turning the receptor on permanently (constitutively), even without signals from outside the cell (Flowchart 6.4).



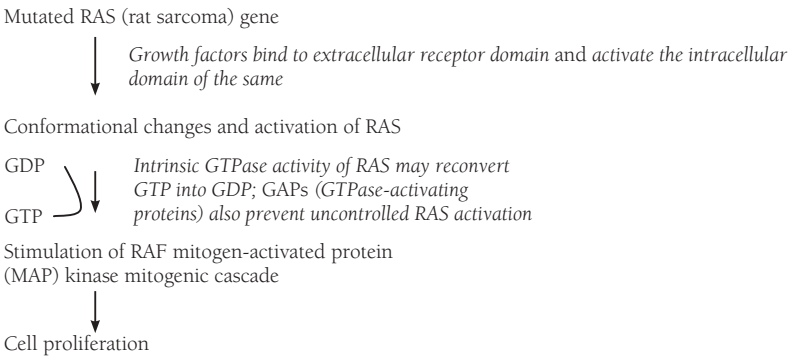
FLOWCHART 6.4. Role of growth factor receptors in evolution of carcinogenesis.

Examples
- Point mutations in ERB-B1 (EGF receptor) found in a subset of lung adenocarcinomas and squamous cell carcinoma result in constitutive activation of EGFR tyrosine kinase.
- Amplification of ERB-B2 results in overexpression of HER2 receptor and its constitutive tyrosine kinase activity leading to carcinomas of breast, lung, ovary and stomach.
- Gene rearrangements may activate other receptor tyrosine kinases, eg, ALK.tyrosine kinase.

(c) *Signal transduction proteins*
- Normal signal transduction proteins, which transduce signals from the growth factor receptors on the cell surface to the nucleus, may get mutated, eg, **mutated *RAS* (rat sarcoma) gene**.
- Point mutations involving RAS family genes (HRAS, KRAS and NRAS; HRAS and KRAS were named after their discoverers, namely Jennifer Harvey and Werner

Kirsten and NRAS was named so as it was initially identified in neuroblastoma cells) are the most common form of abnormality affecting a proto-oncogene in human tumours. As a member of the family of small G proteins, it is mainly implicated in the pathogenesis of carcinoma of colon, lungs and pancreas.

- In the inactive state, RAS proteins bind guanosine diphosphate (GDP); when cells are stimulated by growth factors or other receptor–ligand interactions, RAS becomes activated by exchanging GDP for GTP (guanosine triphosphate; see Flowchart 6.5).
- Activated RAS, in turn, acts on MAP (mitogen-activated protein) kinase pathway by recruiting cytosolic protein RAF-1 (RAF proto-oncogene serine/threonine-protein kinase also known as proto-oncogene c-RAF or simply c-Raf). In addition to MAP pathway, there is activation of the P13K/AKT pathway as well.
- The MAP kinases so activated target nuclear transcription factors, and thus promote mitogenesis.
- In normal cells, the activated signal-transmitting stage of the RAS protein is transient because its intrinsic GTPase activity hydrolyses GTP to GDP, thereby returning RAS to its quiescent ground state.
- Mutations that reduce the GTPase activity of the RAS protein result in an activated GTP-bound form that receives continuous signals.



FLOWCHART 6.5. Role of RAS-signalling pathway in cancer.

(d) Nuclear regulatory molecules: Overexpression of **MYC gene** occurs with t(8;14) in Burkitt lymphoma or may be a result of amplification of the gene as seen in carcinoma of lung, breast and colon (dysregulation due to amplification). Normal MYC protein binds to DNA and regulates the cell cycle by transcriptional activation, and its levels fall immediately after the cell enters the cell cycle. Persistent overexpression of MYC oncogene leads to autonomous cell proliferation.

(e) Cell-cycle regulatory proteins: Normal cell cycle is under control of cyclins and CDK A, B, E and D. Checkpoint is $G_1 \rightarrow S$ phase. Mutations in cyclins (particularly cyclin D) and CDKs (in particular CDK4) may aid in induction of cancer, eg, 'overexpression of cyclin D' is implicated in carcinoma of breast, liver and mantle cell lymphoma, and 'amplification of CDK4' in multiple myeloma, glioblastomas and sarcomas.

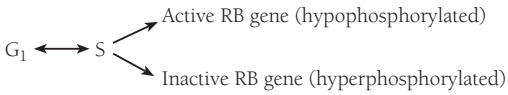
Refractoriness to Growth Inhibition (Loss of Growth-Suppressing Anti-Oncogenes)

Anti-oncogenes or tumour suppressor genes prevent entry of cells in mitotic pool and push cells into the G_0 phase. Mutated anti-oncogenes behave like growth-promoting genes. The following are the important tumour suppressor genes involved in growth inhibition:

1. *Retinoblastoma (RB) gene:*
   - Approximately 60% of retinoblastomas are sporadic, and the remaining 40% are inherited. To explain the inherited and sporadic occurrence of this seemingly identical tumour, **Knudson proposed his now famous '*two-hit' hypothesis of oncogenesis*.**

- He suggested that in **hereditary cases** one genetic change ('first hit') is inherited from an affected parent, and is therefore present in all somatic cells of the body; whereas, the second mutation ('second hit') occurs in one of the many retinal cells (which already carry the first mutation).
- In **sporadic cases**, however, the two mutations (hits) occur somatically within a single retinal cell whose progeny then form the tumour.
- The mutations required to produce retinoblastoma involve the ***RB* gene**, located on **chromosome 13ql4**.
- Both normal alleles of the *RB* locus must be inactivated (two hits) for the development of retinoblastoma.
- In familial cases, children are born with one normal and one defective copy of the *RB* gene. They lose the intact copy in the retinoblasts through some form of somatic mutation (point mutation, deletion of 13q 14 or even complete loss of the normal chromosome 13).
- In sporadic cases, both normal *RB* alleles are lost by somatic mutation in one of the retinoblasts.
- Patients with familial retinoblastoma are also at a greatly increased risk of developing osteosarcoma and some other soft-tissue sarcomas.
- Furthermore, inactivation of the *RB* locus has been noted in several other tumours, including adenocarcinoma of the breast, small cell carcinoma of the lung and bladder carcinoma.
- Most importantly, alterations in the key regulators of the cell cycle or RB pathway (involving INK4a proteins, cyclin D-dependent kinases and RB family proteins) are present in most cancer cells. The RB gene can loose its normal suppressor action and go into the proliferative mode by various mechanisms (loss of function mutations affecting RB; gene amplification of CDK4 and cyclin D; loss of cyclin-dependant kinase inhibitors like p16/INK4 and inhibition of RB by binding of viral oncoproteins to it).

RB pathway:



- The **retinoblastoma gene** encodes a 110-kDa **phosphoprotein (pRB)** that is expressed in almost every cell of the human body and contributes to growth regulation in these cells.
- The most important target of the retinoblastoma protein is cellular transcription factor **E2F**. E2F is a potent stimulator of cell cycle entry into S phase.
- E2F activity consists of an E2F polypeptide and a DP protein (E2F/DP heterodimeric complex).
- In the hypophosphorylated form (which occurs early in G_1), *RB* binds to E2F, inactivates E2F as a transcription factor and shuts the expression of E2F target genes off.
- Phosphorylation of pRB by Cyclin/CDK complexes in mid-to-late G_1 causes pRB to lose its affinity for E2F.
- The free E2F/DP transcription factor can now activate transcription of E2F target genes.
- In a normal cell during mitosis, **phosphatase 1-like protein (PP1)** removes all phosphates from pRB.

2. *Tp53 gene (p53)*: Situated on short arm of **chromosome 17**, it is also called '**protector of the genome**' as *P53* mutation is present in more than half of all human cancers. *P53* has two major functions:
   (a) Blocking mitotic activity: DNA damage is sensed by kinases of the ATM/ATR (ataxia telangiectasia mutated or ataxia telangiectasia and Rad3) family which phosphorylate p53, releasing it from inhibitors like MDM2 (mouse double minute 2 homolog). If the damage is minor, activated p53 enhances expression of CDK inhibitor p21 which arrests/halts the cell-cycle at the G_1/S checkpoint until the damage is repaired.
   (b) Role in promoting apoptosis: If the damage is major and cannot be repaired, *p53* triggers the cell to commit suicide by apoptosis (activates apoptosis, inducing *BAX* gene to bring the defective cell to an end).

Mutated form of *p53* behaves like an oncogene to induce carcinomas of lung, head, neck, colon and breast. It also contributes to sequential development of carcinoma in situ in invasive carcinoma. Cancers of multiple organs (breast, bone and brain sarcomas) are caused by damage to both alleles of p53 (**Li-Fraumeni syndrome**). p53 family has other members like p63 and p73 which show relative tissue specificity unlike p53 which is ubiquitously present.

3. *Transforming growth factor* (TGF-β): Inhibitor of cell proliferation, TGF-β acts on G_1 phase. Its mutant form has impaired-growth-inhibiting effect, leading to uncontrolled cell proliferation and cancer as in carcinoma of pancreas, colon and stomach.
4. *Adenomatous polyposis gene (APC)*:
   - APC is a component of WNT-signalling pathways which controls cell adhesion and polarity during embryogenesis. An important function of the APC protein is to downregulate β-catenin.
   - In the absence of WNT-signalling, APC causes proteasomal degradation of β-catenin by forming a 'destruction complex', thus preventing its accumulation in the cytoplasm. In the event of *APC* gene inactivation, there is disruption of this complex, thereby increasing cellular levels of β-catenin, which, in turn, translocates to the nucleus.
   - In the nucleus, β-catenin forms a complex with TCF-α transcription factor which upregulates cellular proliferation by promoting the transcription of *c-MYC, CYC*LIN Dl and other genes.
   - APC is mutated in familial adenomatous polyposis and sporadic colonic carcinomas. Also, colonic tumours in some cases have normal APC genes but mutated β-catenin, which is not inhibited by APC.
   - Dysregulation of the APC–β-catenin pathway is also present in more than 50% of hepatoblastomas and in approximately 20% of hepatocellular carcinomas.
5. *Wilms tumour (WT)-1 gene*: The *WT-1* gene, located on chromosome l 1p13, is associated with the development of Wilms tumour. It is a transcriptional activator of genes involved in renal and gonadal differentiation. Both inherited and sporadic forms of Wilms tumour occur, and mutational inactivation of the *WT-* I locus have been seen in both forms.
6. *Neurofibroma (NF) gene*: Prevents proliferation of Schwann cells and is involved in neurofibromatosis-1 and -2.
7. *Breast cancer susceptibility genes (BRCA) 1 and 2:*
   - *BRCA*1 gene is located on the long arm of chromosome 17, and its protein product is involved in DNA damage repair and transcriptional regulation. Variations in the gene have been implicated in a number of hereditary cancers, namely breast, ovary and prostate.
   - *BRCA*2 gene is located on the long arm of chromosome 13 and is essential for repairing damaged DNA; the *BRCA*2 protein binds to and regulates the protein produced by the *RAD*51 gene to fix breaks in DNA. Abnormalities of the *BRCA*2 gene may cause an increased risk of breast cancer along with cancer of the ovaries, prostate and pancreas, as well as malignant melanoma.
8. *von Hippel–Lindau (VHL) gene:* Germline mutation of *VHL* gene on chromosome 3p is associated with hereditary renal cell carcinoma, pheochromocytoma, haemangioblastoma of the central nervous system, retinal angiomas and renal cysts. Mutations in *VHL* gene are sometimes also noted in sporadic renal cell cancers.
9. *Phosphatase and tensin homologue (PTEN) gene:* This is located on chromosome 10 and is frequently deleted in endometrial cancer and glioblastoma. *PTEN* activity causes cell cycle arrest and apoptosis as well as inhibition of cell motility. It has been proposed that *PTEN* blocks the cell cycle by increasing the transcription of the p27 Cip/Kip cell-cycle inhibitor and stabilizing the protein. With loss of *PTEN*, the cells continuously replicate.
10. *Cadherins:*
    - Cadherins are a family of transmembrane proteins that play an important role in cell adhesion. They are dependent on calcium ions to function, hence their name. Loss of Cadherins induces loss of cohesion of cells, which then invade locally as well as metastasize.

- Different members of the cadherin family are found in different locations. E-Cadherins are found in epithelial tissue, N-Cadherins are found in neurons and P-Cadherins are found in the placenta.
- Reduced cell-surface expression of E-Cadherins is noted in many cancers, eg, oesophagus, colon, breast and ovary. Germline mutations of E-cadherin gene predisposes to familial gastric carcinomas.

11. *Kruppel-like factor 6 (KLF6):* This encodes a transcription factor that has many target genes, including *TGF*-α and *TGF*-β receptors, and is found to be mutated in more than 70% of primary prostate cancers. It has been proposed that *KLF*6 inhibits cell proliferation by increasing the transcription of the Cip/Kip cell-cycle inhibitor *p21*, independent of *p53*.
12. *Patched (PTCH)*: It encodes a cell membrane protein (PATCHED1), which functions as a receptor for a family of proteins called *Hedgehog*. The Hedgehog–PATCHED pathway regulates several genes, including *TGF*-β and *PDGF*. Mutations in *PTC*H are responsible for **Gorlin syndrome**, an inherited condition also known as nevoid basal cell carcinoma syndrome.
13. *Serine/threonine kinase 11 (STK11):* Also known as LKB1, this encodes a serine/threonine kinase that is a regulator of cellular metabolism. Mutations in STK11 result in Peutz–Jeghers syndrome (benign polyps of GIT and GI and pancreatic carcinomas).

Growth-Promoting Metabolic Alterations

- Cancer cells demonstrate high levels of glucose uptake and increased fermentation of glucose to lactose via the glycolytic pathway even in the presence of adequate oxygen (**Warburg effect**).
- Positron emission tomography (PET scan) uses this glucose hunger of cancer cells to visualize tumour cells. This procedure involves injecting patients with F-fluorodeoxyglucose, which is a nonmetabolizable derivative of glucose and is preferentially taken up by rapidly proliferating tumour cells.
- This preference exhibited for aerobic glycolysis by rapidly proliferating tumour cells over mitochondrial oxidative phosphorylation is because the former provides the tumour cells with metabolic intermediates necessary for synthesis of cellular components not provided by the latter.
- Also, while in the rapidly growing normal cells, aerobic glycolysis stops when the cells are no more proliferating, in cancer cells aerobic glycolysis continues due to enhanced action of oncogenes and decreased action of tumour suppressor genes (owing to progrowth signalling factors like P13K/AKT signalling, upregulated transcription factor MYC and receptor tyrosine kinase activity).

Evasion of Apoptosis

Cancer cells demonstrate abnormalities of both the intrinsic and extrinsic pathways but the former are more commonly encountered, eg, overexpression of anti-apoptotic gene BCL2 in follicular lymphomas.

Stem Cell-Like Replicative Potential

- It has been found that some of the cancer cells behave like stem cells. 'Cancer stem cells' can arise either through transformation of normal stem cells or through genetic aberrations in mature cells which make de-differentiate to push them into a stem cell-like state.
- The ability of 'cancer stem cells' to continuously replicate is attributed to inactivation of senescence signals and reactivation of telomerase.

Q. Write briefly on angiogenesis.

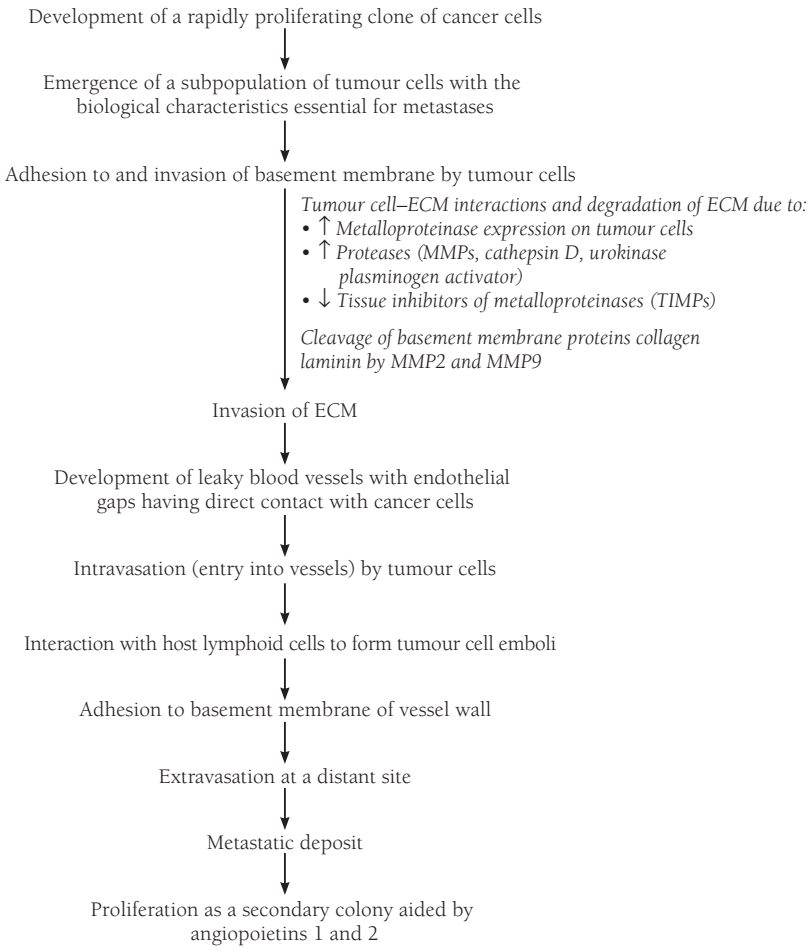
Ans. Neoplasms cannot enlarge beyond 2 mm in diameter unless they undergo neovascularization as the maximal distance across which oxygen and nutrients can diffuse from surrounding blood vessels is 1–2 mm.

- Neovascularization perfuses the tumour and newly formed endothelial cells stimulate the growth of adjacent tumour cells by secreting polypeptide growth factors such as insulin-like growth factors (IGFs) and PDGF.

- Angiogenesis is required not only for tumour growth, but also for metastasis. Without access to the vasculature, the tumour cells cannot spread to distant sites.
- Sprouting of new vessels from already-existing ones is called neoangiogenesis; whereas, vasculogenesis occurs by recruitment of endothelial cell precursors. Tumour blood vessels differ from the normal vasculature by being leaky and unorganized.
- The pro-angiogenic factors are produced by tumour cells or derived from inflammatory cells (eg, macrophages) that infiltrate tumours. Hypoxia activates transcription factor Hypoxia-inducible factor (HIF)-1α which in turn activates pro-angiogenic factors like VEGF and bFGF. Other promoters of angiogenesis include angiopoietins 1 and 2. Inhibitors of VEGF are being used for the treatment of advanced cancers. These are not curative but can prolong survival.
- Under normal circumstances, p53 increases expression of anti-angiogenic factors like thrombospondin-1, angiostatin, tumstatin and endostatin. Loss of p53, therefore, allows angiogenesis.

Q. Describe the mechanism and biology of invasion and metastasis.

Ans. In the early stage of tumourigenesis, the cells are not invasive and metastatic. Progressive genetic aberrations are associated with the appearance of new clones with invasiveness and metastatic ability. Highly metastatic cells often acquire alterations in more genes than nonmetastatic cells. Potentially important genes associated with epithelial metastasis include twist-related protein (TWIST) and the zinc-finger group gene SNAIL; these genes encode transcription factors which increase epithelial-mesenchymal interactions. The following are the steps involved in invasion and metastasis (Flowchart 6.6; Fig. 6.5):



FLOWCHART 6.6. Sequence of events in invasion and metastasis.

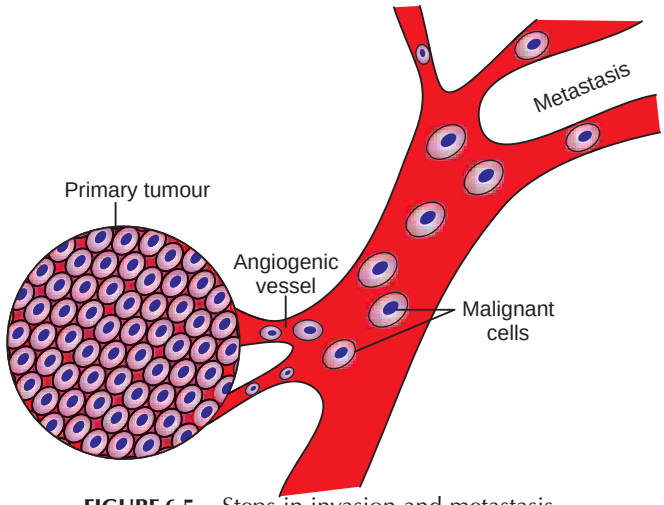


FIGURE 6.5. Steps in invasion and metastasis.

Q. Write briefly on chemical carcinogenesis.

Ans. ***Induction of cancer by chemicals depends on:***

- Dose, duration and mode of administration of the chemical
- Individual susceptibility
- Associated predisposing factors

Stages of Chemical Carcinogenesis

- **Initiation:** results from exposure of cells to sufficient dose of the carcinogen. The change induced is sudden and irreversible, and has memory. Initiation alone, however, is not sufficient for tumour formation.
- **Promotion:** entails proliferation and clonal expansion of the altered and initiated cell. Promoters include phorbol esters, phenols, hormones, artificial sweeteners and phenobarbital. The cellular changes resulting from the application of promoters do not affect DNA directly, and are reversible.

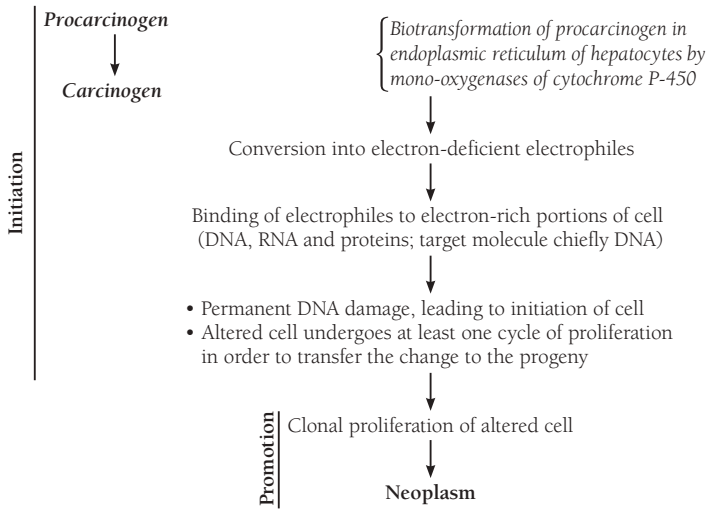
Initiators

1. **Direct-acting carcinogens—**Do not require metabolic activation and include:
   - **(a) Alkylating agents**
     - (i) Anticancer drugs, eg, cyclophosphamide, chlorambucil, busulfan, melphalan and nitrosoureas
     - (ii) β-propiolactone
     - (iii) Dimethyl sulphate
     - (iv) Diepoxybutane
   - **(b) Acylating agents**
     - (i) 1-acetyl imidazole
     - (ii) Dimethyl carbamyl chloride
2. **Indirect-acting procarcinogens—**Require metabolic activation and include:
   - **(a) Polycyclic aromatic hydrocarbons** (found in tobacco, smoke, fossil, fuel, soot, tar, mineral oils and smoked animal foods)
     - (i) Anthracenes (cause lung and skin cancer)
     - (ii) Benzopyrene (cause cancer of oral cavity)
     - (iii) Methylcholanthrene (associated with sarcomas)
   - **(b) Aromatic amines and azo dyes**
     - (i) β naphthylamine (associated with carcinoma of urinary bladder)
     - (ii) Benzidine (role in pathogenesis of hepatocellular carcinoma)
     - (iii) Azo dyes like butter yellow, scarlet red (role in pathogenesis of hepatocellular carcinoma)

(c) Naturally occurring products
- (i) Aflatoxin B_1 (role in pathogenesis of hepatocellular carcinoma)
- (ii) Cycasin (role in hepatobiliary tumours)
- (iii) Safrole (carcinogenic and genotoxic)
- (iv) Betel nuts (oral cancer)

(d) Miscellaneous
- (i) Nitrosamines and amides (role in pathogenesis of gastric carcinoma)
- (ii) Vinyl chloride monomer (implicated in the pathogenesis of angiosarcoma of liver)
- (iii) Asbestosis (may lead to bronchogenic carcinoma and mesothelioma)
- (iv) Nickel, lead, cobalt and chromium (cause epidermal hyperplasia and basal cell carcinoma)

Stages of chemical carcinogenesis are shown in Flowchart 6.7.



FLOWCHART 6.7. Stages of chemical carcinogenesis.

The contrasting features of initiators and promoters are listed in Table 6.7.

TABLE 6.7. Contrasting features of initiators and promoters

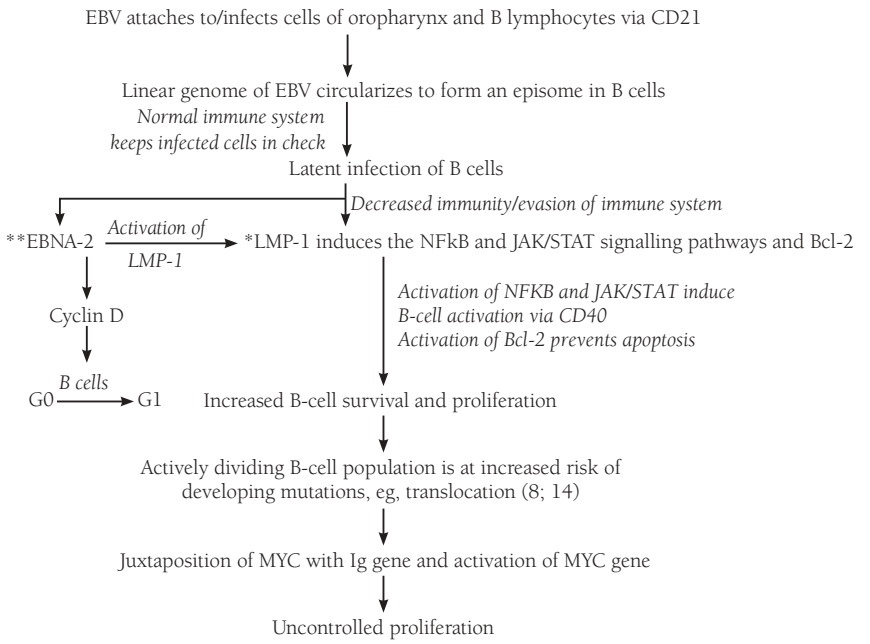
Features	Initiators	Promoters
Sequence of application	Applied first	Applied after initiator
Mechanism	Induction of mutation	Not mutagenic; instead are mitogenic Induce cell cycling and reinforce the action of initiators rather than inducing a mutation
Dose	Single for a short time	Repeated over a long time
Response	Sudden	Delayed
Molecular change	Initiation causes irreversible changes and has memory	Promoters induce reversible changes
Examples	Most chemical carcinogens, radiation	Hormones, phorbol esters

Q. Write briefly on microbial carcinogenesis.

Ans. Carcinogenic microbiological agents mainly include oncogenic DNA and RNA viruses as well as bacteria like *Helicobacter pylori*.

1. **Oncogenic DNA viruses:** Genomes of oncogenic DNA viruses integrate into and forms stable associations with host genome, eg, E6 proteins of high-risk HPV types complex with *p53* to enhance its degradation. Oncogenic DNA viruses include:
   (a) **Human papilloma virus (HPV):** HPV has more than 100 distinct subtypes of which types 1, 2, 4 and 7 cause benign squamous papillomas or warts. Squamous cell carcinomas (SCCs) of cervix and anogenital region, as well as, oral and laryngeal cancers are associated with HPV 16, 18, 31, 33, 35 and 51 and their precursor lesions; whereas, HPV 6 and 11 cause genital lesions with low-malignant potential. HPV genome is present in **episomal (nonintegrated)** form in benign warts and preneoplastic lesions. In cancers, the viral genome appears to be **integrated** into the host DNA. HPV proteins have the following effects on the cell cycle:
      - E6 and E7 block p53 and RB cell-cycle suppression pathways, respectively.
      - E6 proteins of high-risk HPV type complex with p53 to enhance its degradation. E6 proteins of low-risk HPV types have low affinity for p53 and do not affect its stability.
      - Increased p53 degradation causes a block in apoptosis (p53 increases activity of BAX, which is proapoptotic).
      - E7 from high-risk types binds to RB protein, releasing sequestered E2F from the RB–E2F complex, triggering entry of cells in the S phase.
      - E7 from low-risk types has a lower affinity for RB protein, and is slow in transforming cells.
   (b) **Epstein–Barr virus (EBV):** EBV is implicated in the pathogenesis of
      (i) African form of Burkitt lymphoma
      (ii) B-cell lymphoma in immunosuppressed individuals
      (iii) Hodgkin lymphoma
      (iv) Nasopharyngeal carcinoma
      (v) Gastric carcinoma
      (vi) NK cell lymphoma

Mechanism of EBV-induced oncogenesis is shown in Flowchart 6.8.



*LMP-1 – Latent membrane protein-1. **EBNA-2 – Epstein–Barr nuclear antigen 2.

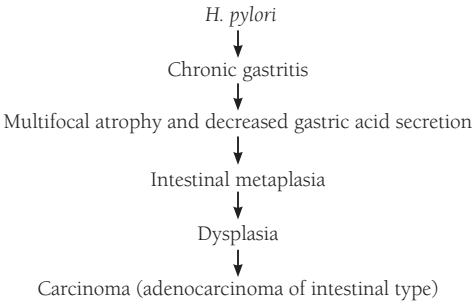
FLOWCHART 6.8. Mechanism of EBV-induced oncogenesis.

(c) **Hepatitis viruses:** 70–80% hepatocellular carcinoma is due to HBV and HCV. Effects of HBV are mainly indirect; it causes chronic liver cell injury, and regenerative hyperplasia (increased pool of cycling cells are at risk for genetic changes). HBV also encodes a regulatory element called **HBX protein,** which disrupts normal growth control of infected liver cells by transcriptional activation of an insulin-like growth factor II and receptors for insulin-like growth factor I. It also binds to P53 and interferes with its growth-suppressing activities.

(d) **Human herpes simplex virus (HHV) 8:** HHV 8 has an established role in Kaposi sarcoma, B cell lymphomas and multicentric variant of Castleman disease. HHV-8 infects host macrophages and primitive mesenchymal cells, which differentiate into endothelial cells under the influence of several cytokines like IL6, IL8 and MIP.

2. **Oncogenic RNA viruses:**
   - Retroviruses are the only RNA viruses that seem to have oncogenic potential in humans. They contain 'reverse transcriptase', which induces reverse transcription of viral RNA to synthesize viral DNA. The viral DNA moves to host cell nucleus and gets incorporated in it. The prototypic example of an oncogenic RNA virus is human T cell leukemia virus (HTLV)-1.
   - HTLV-1 causes adult T cell leukaemia–lymphoma (ATLL) endemic in Japan and Caribbean basin.
   - It has tropism for CD4+ T cells and is transmitted by passage of infected T cells during sexual intercourse, blood product transfusion and breast feeding.
   - It contains *gag*, *pol* and *env* genes typical of other retroviruses. It also contains '*TAX*' gene, which activates several host cell genes involved in proliferation and differentiation of T cells and interferes with DNA repair functions.
3. ***Helicobacter pylori:*** Can be demonstrated in 90% cases of gastritis and 20–30% cases of gastric ulcer, and is also implicated in the pathogenesis of gastric carcinoma and lymphoma (Flowchart 6.9). It induces active B cell proliferation, which predisposes to acquisition of genetic abnormalities, eg, translocation (11; 18).

Differences between DNA and RNA oncogenic viruses are summarized in Table 6.8.



FLOWCHART 6.9. Mechanism of ***Helicobacter pylori***-induced oncogenesis.

TABLE 6.8. Differences between DNA and RNA oncogenic viruses

Features	DNA oncogenic virus	RNA oncogenic virus
Viruses	HPV, EBV, HBV, KSHV	HTLV-1
Genome	Double-stranded DNA	Single-stranded RNA
Reverse transcriptase	Absent	Present
Interaction with host genome	Linear DNA genome forms a double-stranded circle within infected cell and then covalently integrates into the host genome	First RNA is transcribed into DNA, which then integrates into host genome
Name of gene	*Early region A* gene	*src* gene
Name of protein	T antigen	*src* protein
Function of protein	Protein kinase, ATPase activity, binding to DNA and stimulation of DNA	Protein kinase that phosphorylates tyrosine and disturbs the growth control process
Location of protein	Primarily nuclear, but sometimes in plasma membrane	Plasma membrane

Q. Define and classify paraneoplastic syndromes.

Ans. A paraneoplastic syndrome is a symptom complex in patients with cancer that cannot be explained either by local or distant spread of the tumour or by the elaboration of hormones indigenous to the tissue of origin of the tumour.

Salient Features and Classification of Paraneoplastic Syndromes

- Paraneoplastic syndromes are seen in 10–15% patients with cancer and are important to recognize because:
  - They may be the earliest manifestation of occult or hidden cancer in some cases.
  - They may manifest with signs and symptoms due to excessive production of that hormone.
  - They may mimic metastatic disease.
- Paraneoplastic syndromes can be classified into:
  - Endocrinopathies
  - Nerve and muscle syndrome
  - Dermatological disorders
  - Vascular and haematological changes
  - Bone and soft-tissue changes

Endocrinopathies

These are characterized by production of ectopic hormones or hormone-like substances by cells of nonendocrine origin, eg, **Cushing syndrome** caused by ACTH or ACTH-like substances produced by small cell carcinoma of lung, pancreatic carcinoma or neural tumours; **hypercalcemia (most common paraneoplastic syndrome)** caused by excess parathormone or related hormones (TNF-alfa, TGF-beta and IL-1) secreted by squamous cell carcinoma of lung, carcinomas of breast, kidney, ovary and ATLL; and **Carcinoid syndrome** produced by elaboration of serotonin and bradykinin by bronchial adenomas, pancreatic carcinomas and gastric carcinomas.

Nerve and Muscle Syndrome

Examples include immunologically mediated myasthenia gravis in bronchogenic carcinoma, and disorders of the central and peripheral nervous system seen in breast carcinoma.

Dermatological Disorders

Acanthosis nigricans may be a manifestation of carcinoma of the lung, uterus or stomach. Dermatomyositis may be seen in bronchogenic and breast carcinoma.

Vascular and Haematological Changes

Tumour products (usually mucins that activate clotting factors) of pancreatic and bronchogenic carcinoma can induce venous thrombosis **(Trousseau sign).** Nonbacterial thrombotic endocarditis (due to hypercoagulability) is seen in many advanced cancers; and anaemia may develop in association with thymic neoplasms (cause is unknown).

Bone and Soft-Tissue Changes

Hypertrophic osteoarthropathy and clubbing are common presenting symptoms of bronchogenic carcinoma (cause is unknown).

Q. Write briefly on laboratory diagnosis of cancer.

Ans. The modalities for laboratory diagnosis of cancer include:

1. **Cytology**
   The main techniques used for cytological diagnosis are:

   (a) Fine needle aspiration cytology (FNAC) → Direct / Image guided

Air-dried MGG (May–Grünwald–Giemsa)-stained smears or wet (95% ethanol)-fixed–Haematoxylin-and-Eosin stained smears are prepared from the FNAC material obtained and examined.

(b) Exfoliative cytology: Due to loss of cohesiveness, neoplastic cells are continuously shed from tumours into the surrounding space. These are called exfoliated cells and can be examined in the following preparations:
   (i) Papanicolaou smears (for carcinoma cervix)
   (ii) Sputum and bronchial washings (for bronchogenic carcinoma)
   (iii) Pleural, pericardial and peritoneal fluid (for local cancers)
   (iv) Urine (for urothelial malignancies)
   (v) Cerebrospinal fluid (for CNS tumours)
   (vi) Gastric secretions (for gastric carcinoma)

Diagnostic reliability of exfoliative cytology varies between 80% and 97%.

2. **Histopathology:** Histopathological diagnosis entails microscopy supported by clinical and investigative data. Formalin fixation is required for routine histopathology and glutaraldehyde fixation is required for electron microscopy. Frozen sectioning aids in rapid/intraoperative diagnosis.
3. **Histochemistry/cytochemistry (special stains):** These are diagnostic tools for identifying **chemical composition of cells for the purpose of tumour diagnosis and classification** (Table 6.9).

TABLE 6.9. Special stains in tumour diagnosis

Substances	Stain
Basement membrane/collagen	PAS
	Reticulin
	Van Gieson
	Masson's trichrome
Glycogen	PAS with diastase loss
Glycoproteins	PAS
Mucins of epithelial origin	Mucicarmine
Acid mucins (of mesenchymal origin)	Alcian blue
Argyrophilic/argentaffin granules/fungi	Silver stains
Fat	Oil red-O, Sudan black B

4. **Immunohistochemistry/immunocytochemistry:** Immunohistocytochemistry (IHC) is an immunological method of recognizing a cell based on recognition of specific components called 'antigens'. 'Specific antibodies' against antigens are raised by hybridoma technique and labelled monoclonal antibodies. Antigen–antibody complexes on the slides (histological sections or cytology smears) are made visible for microscopic identification by labels (fluorochromes or enzyme systems).

Uses

- Categorization of undifferentiated neoplasms
- Specific typing of leukaemias/lymphomas
- Determination of site of origin of a metastatic tumour
- Detection of molecules that have *prognostic* or *therapeutic* significance, eg, ER–PR receptors in carcinoma breast
- Expression of protein products of oncogenes
- Differentiating benign from malignant lesions

5. **Intermediate filaments (IFs):** IFs are a family of related proteins that share common structural features. They have an average diameter of 10 nanometers, which is between that of microfilaments (which are smaller) and microtubules (which are larger). Most types of intermediate filaments are cytoplasmic except lamins, which are nuclear. The most important function of intermediate filaments is to provide mechanical support for the plasma membrane, where they come in contact with other cells or with the extracellular matrix. Unlike

microfilaments and microtubules, intermediate filaments do not participate in cell motility. In higher vertebrates, the subunits composing intermediate filaments constitute a superfamily of highly α-helical proteins that is divided into subtypes on the basis of similarities in sequence (Table 6.10).

TABLE 6.10. 'Intermediate filaments' and their significance in the tumour diagnosis

Keratins	Carcinomas, mesotheliomas and germ-cell tumours
Vimentin	Sarcomas, melanomas and lymphomas (mesenchymal tumours)
Desmin	Myogenic tumours
Neurofilaments	Neural tumours
Glial fibrillary acidic proteins	Glial tumours

6. **Electron microscopy (EM):** EM is used for confirming or substantiating tumour diagnosis based on:
   (a) Presence and type of cell junctions
   (b) Presence of microvilli
   (c) Shape of nucleus, features of nuclear membrane and nucleoli
   (d) Cytoplasmic organelles
   (e) Presence of dense bodies in the cytoplasm
7. **Tumour markers:** These are substances found in blood, urine, body fluids or tissue, the levels of which might be elevated in association with a cancer. Tumour markers may be used to help diagnose cancer, predict a patient's response to cancer therapy, check a patient's response to treatment or determine cancer recurrence. More than 20 tumour markers are currently in use (Table 6.11).

TABLE 6.11. Role of tumour markers in neoplasia

Tumour marker	Associated neoplasm
AFP (α-fetoprotein)	Hepatocellular carcinoma, nonseminomatous-germ-cell tumours
PSA (prostate-specific antigen)	Prostatic carcinoma
HCG (human chorionic gonadotropin)	Trophoblastic tumours
Calcitonin	Medullary carcinoma of thyroid
Vanillylmandelic acid (VMA)	Pheochromocytoma
CA-125	Carcinoma of ovary
CEA (carcinoembryonic antigen)	Cancer of bowel, pancreas and breast
CA-15.3	Carcinoma of breast

Modern Aids in the Tumour Diagnosis

1. **Flow cytometry:** Recognition and quantification of several parameters simultaneously by making single-cell suspensions of cells, which are made to pass through a chamber in a single file. Fluids, blood and bone marrow can be processed directly; whereas, homogenization is necessary for solid tissue. Each cell is struck by a focused laser beam, and the properties of scattered and fluorescent light is measured to characterize the cell.

Material is analyzed for → DNA content (aneuploidy is associated with poor prognosis)
Material is analyzed for → Identification of cell surface antigens

2. **In situ hybridization:** Molecular technique by which nucleic acid sequences (cellular/viral DNA and RNA) are localized by specifically labelled nucleic acid probes *directly in the cell* rather than after DNA extraction, eg, localization of oncogenes.
3. A variety of DNA-/RNA-based techniques in which DNA/RNA are extracted and analysed, eg, **DNA analysis by Southern blot and RNA analysis by Northern blot** are also available.
4. A molecular cytogenetic technique called **spectral karyotyping** is a highly sensitive method that allows the examination of all chromosomes in a single experiment. This technique, which is based on 24-colour chromosomal painting with a mixture of fluorochromes, can detect all types of chromosomal rearrangements in tumours including very small translocations and insertions.
5. Another available technique, **comparative genomic hybridization,** is more frequently used in research laboratories as it requires a lot of time and effort. This technique allows the analysis of genome amplification and chromosomal gains and losses in tumour cells. It has been used to differentiate primary from metastatic carcinomas, and to identify primary tumours of uncertain origin.
6. **DNA microarray analysis and proteomics:** These methods are used to obtain gene expression signatures (molecular profiles) of cancer cells. DNA microarray techniques reveal the RNA expression from as many as 30,000 different genes using gene–chip technology. Conventional DNA probes are substituted by silicon chips that contain the entire range of genes, and high-resolution scanners are used for measurement. *Proteomics* determines the protein profiles of tumours. With the methods currently in use, protein profiles from about 3,000 genes can be obtained.
7. Validation of new markers for cancer diagnosis can be done on multiple tissue samples, using **tissue arrays.** In this technique, core samples are obtained from tissues embedded in a paraffin block and used to prepare a new block that may contain hundreds of tissue fragments. These multiple samples are then used to test the expression of potential tumour markers by immunohistochemical or in situ hybridization techniques.

The above molecular methods can be used for

- **Analysis of molecular cytogenetic abnormalities and mutational analysis:** Certain genetic alterations are associated with poor prognosis, and hence their detection allows stratification of patients for therapy. As an example, amplification of the *N-MYC* gene and deletions of 1p bode poorly for patients with neuroblastoma. These can be detected by routine cytogenetics, and also by fluorescent in-situ hybridization (FISH) or polymerase chain reaction (PCR) assays.
- **Study of oncogenic viruses at molecular level:** Oncogenic viruses can contribute to different steps of the carcinogenic process. In addition to elucidate the aetiology of several human cancers, the study of oncogenic viruses has been invaluable to the discovery and analysis of key cellular pathways that are commonly rendered dysfunctional during carcinogenesis, in general.
- **Detection of minimal residual disease:** After treatment of patients with leukaemia or lymphoma, the presence of minimal disease or the onset of relapse can be monitored by PCR-based amplification of unique nucleic acid sequences generated by the translocation. For example, detection of *BCR-ABL* transcripts by PCR gives a measure of the residual leukaemia cells in treated patients with chronic myeloid leukaemia.
- **Diagnosis of hereditary predisposition to cancer:** As was discussed earlier, germline mutations in several tumour suppressor genes, including *BRCA*1, *BRCA*2 and the *RET* proto-oncogene are associated with a high risk of developing specific cancers. Thus, detection of carriers of these mutations in family members of affected patients or in those at high risk of carrying the mutation can be achieved by molecular methods.

7

Infections

General terminology pertaining to infectious diseases

- **Infectious diseases** are **transmissible** or **communicable diseases** that can spread directly (person to person) or indirectly (through a mediator, eg, air, water, food, living vector or an object-vehicle).
- They are caused by pathogenic micro- or macro-organisms, such as bacteria, viruses, parasites or fungi and have characteristic **'symptoms** and **signs'** resulting from the introduction of the pathogenic biological agent into the host body and its subsequent multiplication. In some cases, infectious diseases may not be clinically evident (are asymptomatic) for most or their entire course.
- The term **'infection'** may not necessarily indicate **'disease',** as some infections lie dormant in the host without causing visible illness.
- The term **infectivity** indicates the ability of an organism to gain entry into and proliferate in the host, while **infectiousness** of a disease indicates the rate at with which it is transmitted to other hosts.
- A **contagious disease** is a type of infectious disease that is easily transmitted by contact. Infectious diseases with more specialized routes of infection, such as vector or sexual transmission, are usually not regarded as 'contagious', and often do not require **medical isolation** (**quarantine**) of victims.
- **Virulence** is defined as the likelihood of an organism causing severe or aggressive disease (degree of pathogenicity).
- **Primary pathogens** are those which cause disease in a normal, healthy host, due to their inherent virulence.
- Organisms that cause disease in an immunosuppressed host are termed as **opportunistic pathogens**. These may be a part of normal host flora (*Candida*) or may be acquired from the environment (eg, introduction via surgical or traumatic wound infections).
- **Zoonotic diseases** are infectious diseases of animals that can be transmitted to humans through animal contact, bite, secretions or vectors.
- **Epidemiology** is a tool used to study disease in a population. It is important to determine whether an infectious disease outbreak is **sporadic** (has an occasional occurrence), **endemic** (occurs regularly at a certain frequency in a region), **epidemic** (occurs at an unusually or unexpectedly high frequency in a particular region) or **pandemic** (occurs worldwide or globally as an epidemic).
- Based on the type of causative agent, infections are classified into those caused by **bacteria, viruses, fungi, protozoa, chlamydiae, rickettsia, mycoplasma** and **helminths.**

Q. Write in detail on various types of bacterial infections.

Ans. Common bacterial infections include

Staphylococcal Infections

- Rosenbach in 1884 described two pigmented colony types of staphylococci and called them: *Staphylococcus aureus* (yellow) and *Staphylococcus albus* (white). The latter later came to be known as *Staphylococcus epidermidis.*

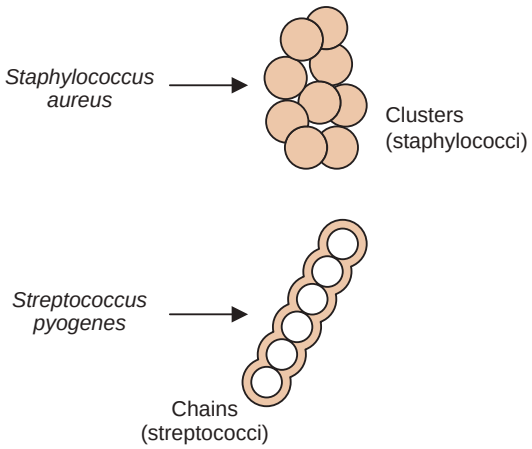


FIGURE 7.1. Bacterial arrangements.

- *S. aureus is a* pyogenic, nonmotile, Gram-positive bacterium that forms grape-like clusters (Fig. 7.1). It is mainly found in the nasal passages, but may also inhabit the skin, oral cavity and gastrointestinal tract. It is considered the most virulent of the more than 30 known pathogenic staphylococcal species. The remaining species of staphylococci are collectively labelled *coagulase-negative staphylococci* and are important pathogens in infections associated with implants and prosthetic devices. *S. epidermidis* is a skin commensal associated with opportunistic infections. *S. saprophyticus* is a common cause of urinary tract infections.
- *S. aureus* produces membrane damaging or haemolytic toxins including α-toxin (intercalates into plasma membrane to form pores); β-toxin (a sphingomyelinase); γ-toxin (lyses RBCs) and δ-toxin (detergent-like peptide). It also produces exfoliative toxins A and B which are serine proteases that cleave desmoglein-1 (aprotein that holds the keratinocytes together) to detach keratinocytes from one another and from the basement membrane (responsible for impetigo and staphylococcal scalded skin syndrome [SSSS]).

Clinical Manifestations

- *S. aureus* is a common cause of **wound infections, respiratory tract infections, lung abscess, empyema** (pus in the pleural cavity)**, sinusitis, otitis media, breast abscess, umbilical sepsis osteomyelitis, endocarditis, pericarditis and bacteraemia,** ocular infections including **conjunctivitis** and **endophthalmitis,** infection of the nail bed **(paronychia)** and most **hospital-acquired infections**.
- It is a major cause of invasive infections of the skin such as **folliculitis** (infection of hair follicles), formation of **furuncles** (boils in the hairy, moist regions of the body) and **carbuncles** (suppurative collection in the lower neck reaching up to the subcutaneous tissue), **abscesses, impetigo** (superficial infection of the skin), **cellulitis** (infection of deeper layers of skin and subcutaneous tissue), **lymphadenitis** and **hidradenitis suppurativa** (chronic abscess formation in apocrine gland regions, most frequently axillae)**.**
- **Toxic shock syndrome (TSS)**, **food poisoning, SSSS** and **necrotizing pneumonia** are the other manifestations of *S. aureus* infection. **TSS,** which is due to superantigens of *S. aureus,* is usually seen in tampon-wearing menstruating women and patients with infected surgical wounds. Its clinical features include high fever, mental confusion, diarrhoea, hypotension, pharyngitis and an erythematous rash that occur during or soon after menses. The rash occurs predominantly on the hands and feet, and resolves with desquamation in 7–10 days. **SSSS** (also called **Ritter disease)** is attributed to the staphylococcal exotoxins A and B, which lead to an exfoliative dermatitis that most frequently follows nasopharyngeal and skin infections in children.

Morphology

S. aureus causes pyogenic inflammation with a tendency for local destruction. **Histopathology** usually shows deep-seated suppuration that tends to expand laterally to form multiple sinuses in the adjacent skin.

Streptococcal Infections

- Streptococci are Gram-positive pathogens that divide in a single plane and thus occur in pairs or chains of varying lengths (Fig. 7.1).
- *Streptococcal species* are classified based on their haemolytic properties. β-haemolytic streptococci can be further subclassified based on the antigenic differences in group-specific polysaccharides (Lancefield antigens) located in the bacterial cell wall (designated by letters A, B and C).
- *Streptococcus pyogenes* is a Group A (beta-haemolytic) streptococcus that colonizes throat or skin. It is covered by a hyaluronic acid capsule and a layer of carbohydrate and is an important cause of many invasive and noninvasive infections.
- Amongst the α-haemolytic streptococci, *Streptococcus pneumoniae* is the most important. It is a well-known cause of community-acquired pneumonia and meningitis in adults. *Streptococcus viridians,* another α-haemolytic streptococcus, are not only part of normal oral flora but are also a common cause of endocarditis. *Streptococcus mutans* is major cause of dental caries.
- *Streptococcus pyogenes and Streptococcus pneumoniae* have capsules that are resistant to phagocytosis. *S. pyogenes* also has M protein which contributes to its resistance to phagocytosis and a pyogenic exotoxin that is responsible for fever and rash in scarlet fever.

Clinical Manifestations

- Otherwise part of normal flora, in immunosuppressed individuals, *S. pyogenes* can cause a variety of suppurative infections such as **puerperal sepsis, pharyngitis** (formation of microabscesses in the tonsillar crypts), **erysipelas** (infection of the dermal lymphatics characterized by rapidly spreading erythematous cutaneous swelling), **impetigo** or **cellulitis.**
- **Scarlet fever** (streptococcal pharyngitis with an erythematous rash) is caused by a strain of *S. pyogenes* which produces the *'erythrogenic toxin'*. The toxin induces a rash having a sandpaper consistency that originates on the trunk and limbs, and resolves with desquamation. Accompanying the rash are some changes in tongue (initially 'white-strawberry' followed by a 'red-strawberry' appearance).
- Acute streptococcal infection may sometimes result in **immune-mediated sequelae**, such as **acute rheumatic fever** and **acute glomerulonephritis** (antistreptococcal M protein antibodies and T cells cross react with cardiac and renal proteins).
- Invasive infection with toxin-producing strains may result in **necrotizing fasciitis**, **muscle necrosis** and **streptococcal toxic shock syndrome (TSS)**.
- *S. pneumoniae* is an important cause of **lobar pneumonia** (produces toxin 'pneumolysin' which causes membrane lysis and tissue destruction).

Morphology

Although streptococcus causes lesions similar to those caused by staphylococcus **(furuncles, carbuncles** and **impetigo)**, it shows lesser fewer tendency for destruction. Classic histopathological finding is diffuse interstitial neutrophilic infiltrate.

Diphtheria

Diphtheria is caused by ***Corynebacterium diphtheria,*** a Gram-positive, aerobic, non-motile, rod-shaped bacteria with clubbed ends classified as ***Actinobacteria,*** which undergo snapping movements just after cell division resulting in characteristic Chinese letter like forms. It is transmitted from person-to-person through aerosols/skin shedding.

Clinical Manifestations

- *C. diphtheriae* infection may be **asymptomatic** or manifest as **clinical diphtheria**. The latter may be classified as **nasopharyngeal** or **cutaneous** depending on the area of involvement.
- **Pharyngeal diphtheria** has a wide spectrum of clinical manifestations ranging from mild pharyngitis to airway obstruction due to the formation of a pseudomembrane. The bacteria induce the formation of an intense fibrinosuppurative exudate, the coagulation of which creates a tough, dirty, grey membrane, which eventually leads to asphyxiation. Accompanying cervical lymphadenitis causes marked swelling of the neck (also called bull neck diphtheria). Released toxins can cause loss of motor function leading to serious complications, eg, inability to swallow and congestive heart failure (also attributed to direct action of diphtheria toxin on the myocardium).
- Infection of chronic wounds is a common manifestation of **cutaneous** diphtheria. The skin lesions are also covered by a grey-brown pseudomembrane like the pharyngeal lesions.

Morphology

Histological sections typically show abundant neutrophils, vascular congestion, interstitial oedema and fibrin exudation. The release of exotoxins induces generalized hyperplasia of the reticuloendothelial system, degeneration of myelin sheaths of nerves, fatty change and necroses of multiple organs such as the myocardium, liver, kidneys and adrenals.

Anthrax

Anthrax is a zoonotic infection caused by *Bacillus anthracis,* which is a spore-forming, Gram-positive, rod-shaped bacterium. It occurs in animals that have contact with soil contaminated with *B. anthracis* spores. Anthrax spores can be ground to a fine powder which makes them a potential weapon for bioterrorism. *B. anthracis* produces potent toxins and has a polyglutamyl capsule, which is antiphagocytic. There are three major anthrax syndromes:

1. **Cutaneous anthrax:** Responsible for 95% cases of anthrax, cutaneous anthrax begins as a painless itchy papule which eventually transforms into a vesicle. The cutaneous lesion is accompanied by regional lymphadenopathy. The vesicle ruptures to form an ulcer that gets covered with dead tissue (eschar). Shedding of the eschar is a sign of recovery. Bacteraemia is rarely seen. *Histopathology* of anthrax skin lesions shows oedema, necrosis and lymphocytic infiltration. No suppuration is seen. *Gram's staining* demonstrates bacilli in the subcutaneous tissue.
2. **Inhalational anthrax:** Occurs due to inhalation of anthrax spores, which then travel to the regional lymph nodes via macrophages. The anthrax spores germinate in the lymphatics and release toxins. This results in high-grade fever, cough, chest pain, breathlessness, excessive sweating, shock and frequently death. *Histopathology* shows necrotizing haemorrhagic pneumonitis, submucosal haemorrhages in the respiratory passages, with haemorrhage and necrosis of peribronchial lymph nodes. Gastrointestinal and meningeal lesions may occur as a result of haematogenous spread.
3. **Gastrointestinal anthrax:** This is the least common form of anthrax. It is introduced into a human via contaminated undercooked meat. Manifestations include flu-like symptoms (fever, fatigue and sore throat); neck swelling, difficulty in swallowing, abdominal pain, vomiting and diarrhoea (both of which may be bloody). *Microscopy* reveals massive oedema, lymphocytic infiltrate and necrosis at infected sites. *Gram's staining* of peritoneal fluid may demonstrate gram-positive bacilli.

Plague

- It is a zoonotic infection caused by *Yersinia pestis*, a Gram-negative, facultative, intracellular bacterium, transmitted by fleabites or aerosols. It has an incubation period of 2–7 days. The disease is frequently fatal (thus named 'Black Death').

- **The three most common forms of plague include**
  - Bubonic plague (affects the lymphatic system)
  - Pneumonic plague (affects the respiratory tract)
  - Septicaemic plague (infection of blood)
- Rodents, such as rats, are carriers of the disease and plague accidentally affects humans when they are bitten by a flea that carries the plague bacteria from an infected rodent. Rarely, one may get the disease while handling an infected animal. Pneumonic plague can spread from human-to-human via respiratory droplets.

Morphology

- The distinctive pathological features of plague include protein-rich effusions, marked tissue swelling, necrosis with haemorrhage and thrombosis and massive neutrophilic infiltrates.
- **Bubonic plague** usually initiates on the legs as a small pustule or ulcer. This enlarges to involve the draining lymph nodes which become soft and pulpy, and may rupture through the skin.
- **Pneumonic plague** typically presents with severe necrotizing bronchopneumonia, often accompanied by haemorrhage and fibrinous pleuritis.
- Disseminated necrotizing lymphadenitis is the histopathological hallmark of **septicaemic plague.** Bacteraemia may induce disseminated intravascular coagulation (DIC) with the presence of widespread haemorrhages and thrombi.

Plague can be diagnosed by

- Blood culture
- Culture of lymph node aspirate (bubo aspirates)
- Sputum culture (in pneumonic plague)

Typhoid Fever

Also known as **'enteric' or 'bilious fever', typhoid** is caused by the Gram-negative bacillus, ***Salmonella typhi.*** The extent and severity of clinical disease depends on the bacterial type and its strain. Salmonella possesses **protective antigens** which promote host destruction; these include a heat-stable cell wall lipopolysaccharide (LPS) known as **somatic or 'O' antigen, flagellar or H antigens** derived from structural proteins and a **PS capsular Vi (**for virulence**)** antigen found at the surface of freshly isolated strains.

Pathogenesis

- Typhoid is transmitted by ingestion of food or water contaminated with faeces from an infected person.
- After reaching the lumen of intestine, the bacteria multiply by attaching to microvilli of the intestinal surface. They eventually perforate through the intestinal wall and are phagocytosed by macrophages. *S. typhi* alters its structure to resist destruction and allows it to exist within the macrophage.
- The bacteria localize in the Peyer's patches in ileum inducing their hyperplasia. Overt enlargement of the Peyer's patches causes ulceration of the overlying mucosa. The organism may spread via lymphatics to get access to reticuloendothelial system and then disseminate throughout the body.

Clinical Manifestations (Table 7.1)

Typhoid is characterized by a sustained, slowly rising fever accompanied by profuse sweating, diarrhoea, a rash of flat rose-coloured spots, tender hepatosplenomegaly and elevation of liver transaminases. Less commonly, there is relative bradycardia, malaise, headache, cough, rarely epistaxis, abdominal pain and delirium. The illness classically lasts for 3–4 weeks. By the end of third week, recovery commences.

TABLE 7.1. Clinical manifestations of typhoid

Disease period	Signs and symptoms	Pathology
First week	Fever, chills, headache, abdominal tenderness	Bacteraemia
Second week	Rash, diarrhoea or constipation, hepatosplenomegaly	Hyperplasia of ileal Peyer's patches and typhoid nodules in spleen and liver
Third week	Complications of intestinal bleeding and perforation, shock, melena, ileus, coma	Ulceration over Peyer's patches, perforation with peritonitis, septicaemia
Fourth week	Resolution of symptoms/relapse, cholecystitis, chronic faecal carriage of bacteria	—

Complications

- Bleeding from congested Peyer's patches or eroded vessels in ulcer base
- Perforation in distal ileum is frequently fatal and may be followed by septicaemia and peritonitis
- Metastatic abscesses in other organs
- Osteomyelitis, endocarditis, glomerulonephritis and infection of genitourinary tract or meningitis
- *S. typhi* preferentially localizes in the gall bladder, where infection tends to become chronic, especially in individuals with a pre-existing pathology

Morphology

- Ileum shows superficial, longitudinal mucosal ulcers aligned along Peyer's patches.
- Intestinal wall shows chronic nonspecific inflammation with numerous macrophages and prominent erythrophagocytosis.
- Draining lymph nodes show reactive hyperplasia and the liver may show focal hepatocytic necrosis with the replacement of the parenchyma by macrophage aggregates called '**typhoid nodules**'.

Diagnosis

- Peripheral blood shows leukopenia with relative lymphocytosis. Rarely thrombocytopenia may be seen.
- *Salmonella* species can be isolated from blood during the first week of fever and from stool or urine in the second or third week.
- 'Widal test' is positive after the first week. It is a serological test which involves demonstration of agglutinating antibodies against O-somatic and H-flagellar antigens in the blood of the affected individual. Cross-reactivity can be seen with antibodies formed against other bacteria and this can result in a false-positive result. False positive results are also possible in the event of typhoid vaccination, and general level of antibodies in endemic areas (therefore rising titer is more important and a value >1:160 is convincing).
- *'Typhidot'* is a rapid test used to diagnose typhoid fever, and is negative in the first week and positive thereafter.
- Indirect haemagglutination test, indirect fluorescent antibody test, indirect enzyme-linked immunosorbent assay (ELISA) for IgM and IgG antibodies to *S. typhi* polysaccharide, and monoclonal antibodies against *S. typhi* flagella (STF) have variable success rates as per existing literature.

Neisserial Infections

- *Neisseria* are Gram-negative diplococci with flattened adjacent sides giving the pair the shape of a coffee bean. They are aerobic (grow best on enriched media such as lysed sheep's blood agar or 'chocolate' agar).

- There are two clinically significant Neisserial species:
  - *N. gonorrhoeae* (*gonococcus*): Causes gonorrhoea
  - *N. meningitides* (*meningococcus*): Causes meningitis
  - *N. gonorrhoeae* normally colonizes mucosal surfaces. Humans are the only host and transmission is via sexual contact. The bacteria need to have fimbriae (pili) to be virulent as the latter enable the gonococcus in attaching to host cells.
  - *N. meningitides* normally inhabits the human nasopharynx and can sometimes overcome the body defences to cause meningitis and septicaemia. Its virulence is mainly due to its antiphagocytic capsule and meningococcal LPS.

Clinical Manifestations

- *N. gonorrhoeae* causes painful urethritis in men. In women, the infection is often asymptomatic and so might go untreated. Untreated chronic infection can lead to pelvic inflammatory disease, which in turn can cause infertility or ectopic pregnancy. Disseminated infection can cause septic arthritis accompanied by a haemorrhagic rash.
- *Neisseria meningitidis* causes meningitis and meningococcemia, a life-threatening sepsis.

Chancroid (Soft Chancre)

Chancroid is a sexually transmitted infection caused by *Haemophilus ducreyi,* a small, Gram-negative and facultative anaerobic bacillus that is highly infective. It is one of the most common causes of genital ulcers in Southeast Asia, where it probably serves as an important cofactor in the transmission of HIV-1 infection. It is known to spread to other anatomical sites by autoinoculation.

Clinical Features

- The disease has an incubation period of 5–7 days. It typically begins as a small inflammatory papule at the site of inoculation; within days, the papule may erode to form an extremely painful deep irregular ulceration.
- In males, the primary lesion is usually located on the penis; in females, most lesions occur in the vagina or the periurethral area. Unlike the primary chancre of syphilis, the ulcer of Chancroid is not indurated and multiple lesions may be present.
- Regional lymphadenopathy is very common. In untreated cases, inflamed nodes may erode overlying skin to produce chronic, draining ulcers.

Morphology

- Microscopically, the ulcer of Chancroid contains three zones, a superficial zone of neutrophilic debris and fibrin; a middle zone of granulation tissue and oedema; and a deep zone of lymphoplasmacytic inflammation. Coccobacilli can sometimes be demonstrated by Gram or silver stains.
- *H. ducreyi* is a fastidious bacterium requiring a relatively expensive nutritive base to grow on and is an extremely difficult organism to culture from clinical specimens. DNA amplification techniques have shown a somewhat improved diagnostic sensitivity but are only performed in a few laboratories.

Granuloma inguinale

Granuloma inguinale (***donovanosis***) is a sexually transmitted, chronic inflammatory disease caused by *Klebsiella granulomatis*, formerly *Calymmatobacterium granulomatis*, a Gram-negative rod. It is endemic in tropical and subtropical climates.

Clinical Features

- The average incubation period varies between 2 and 4 weeks.
- The initial lesion may be a papule, a subcutaneous nodule or an ulcer, which weeks later converts into a raised, soft, painless, beefy-red, superficial ulcer with characteristic rolled edges.

- The lesion spreads by peripheral extension and may have satellite lesions (pseudo buboes). It is generally not accompanied by inguinal lymphadenopathy.
- Untreated cases are characterized by development of extensive scarring, often associated with lymphatic obstruction and lymphoedema (elephantiasis) of external genitalia.

Morphology

- Main pathology is an ulcer accompanied by abundant granulation tissue, which on gross examination appears as a soft, fleshy, painless mass.
- Active lesions are marked by epithelial hyperplasia **(pseudoepitheliomatous reaction).** A mixture of neutrophils and mononuclear inflammatory cells is usually present at the base of the ulcer.
- Culture of the organism is difficult, so morphologic examination of smears or biopsy are the mainstay of the diagnosis.

Tuberculosis

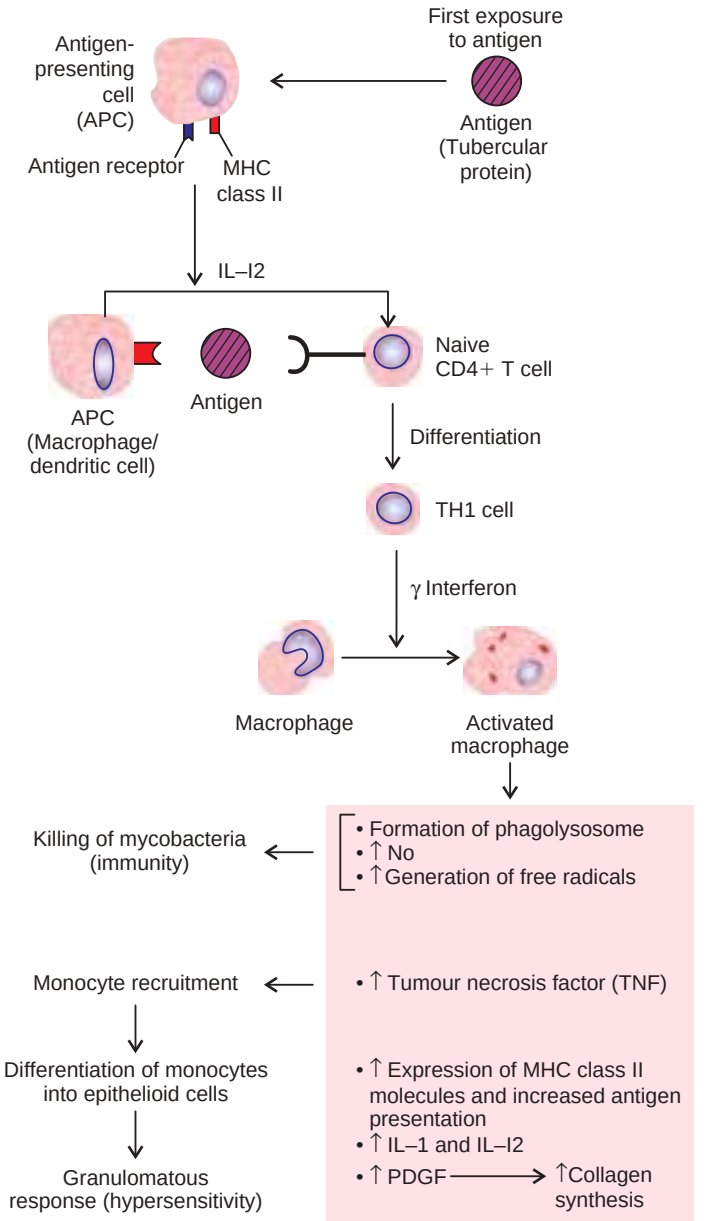
- It is caused by *Mycobacterium tuberculosis,* a slender, aerobic rod which belongs to the genus *Mycobacterium.*
- *Mycobacteria* possess a waxy cell wall composed of mycolic acid, which makes them *acid fast.*
- 'Infection' with *M. tuberculosis* must be differentiated from 'disease'. Infection indicates mere presence of the pathogenic organisms, which may or may not cause clinically significant disease.
- Mycobacteria spread from person-to-person via airborne droplets containing organisms from an active case to a susceptible host.
- Primary tuberculosis is usually asymptomatic; although it may sometimes cause fever and pleural effusion. The primary focus undergoes spontaneous healing by fibrosis and/or calcification in most individuals; however, progression of the disease can occur in a few.
- Viable organisms may remain dormant in such lesions for decades. Reactivation of the infection occurs when the person's immune defences are lowered.

Pathogenesis

- The entry of *M. tuberculosis* into **macrophages** occurs through endocytosis and is influenced by several macrophage receptors such as **mannose receptors** (that bind lipoarabinomannan or LAM) and **complement receptors** (that bind the opsonized organisms).
- Mycobacteria replicate within the macrophage and block formation of phagolysosome by **inhibition of calcium signals** as well as **recruitment and assembly of proteins that cause formation of the phagolysosome** (Flowchart 7.1).

Primary Tuberculosis

- Primary tuberculosis develops in individuals who are previously unexposed or unsensitized to M. tuberculosis.
- Initially, only a nonspecific inflammatory reaction is evident, followed 2–3 weeks later by a positive skin test, which is due to a specific granulomatous parenchymal response. The latter manifests as a tubercle which could be with or without caseation.
- Primary tuberculosis can involve the following sites:
  - Lung
  - Intestine
  - Skin
  - Oropharynx
  - Lymphoid tissue/tonsil
- In areas of high tuberculosis transmission, primary pulmonary tuberculosis has a high incidence in children. Most commonly involved areas are the middle and lower lung zones because most inspired air is distributed to them.



FLOWCHART 7.1. Sequence of events in primary tuberculosis.

- **The lesion forming after primary infection is called 'primary complex' and it has the following components:**
  a) ***Parenchymal component (Ghon focus)***: A subpleural, 1–1.5 cm parenchymal lesion, often located just above or below the interlobar fissure (between the upper and lower lobes).
  b) ***Lymphatic component***: Enlarged lymph nodes and lymphatics draining the parenchymal lesion.
- **Fate of primary infection:**
  - Most primary lesions are asymptomatic and heal spontaneously by undergoing fibrosis and calcification.
  - In infants, children and immunodeficient individuals, primary tuberculosis may progress to **progressive primary tuberculosis** (Flowchart 7.2).

Pneumonia-like consolidation of middle and lower lobes,
hilar lymphadenopathy and pleural effusion (cavitation is rare)

↓

Lymphatic or haematogenous dissemination

↓

Tuberculous meningitis and miliary tuberculosis

FLOWCHART 7.2. Progressive primary tuberculosis.

Secondary Tuberculosis

- Secondary tuberculosis occurs due to reinfection or reactivation of a silent primary focus in a previously sensitized individual.
- Mostly involves apex of one or both lungs **(Simon focus).** Due to pre-existing hypersensitivity, the bacilli induce a rather prominent and rapid response which walls off the infective focus.
- Localized, apical, secondary pulmonary tuberculosis may heal with fibrosis either spontaneously or after therapy (usual outcome in an immunocompetent host), or the disease may progress and extend along several different pathways (in the elderly or the immunocompromised).
- Secondary tuberculosis is typically characterized by less nodal involvement and more cavitation (**cavitary tuberculosis).** The apical lesion enlarges and drainage of caseous necrosis into a bronchus creates a cavitary lesion.
- Cavitation can lead to rupture of vessels within it, thus presenting with **haemoptysis.**
- Also, cavitation aids in spread of disease by haematogenous, lymphatic or contiguous routes and can have one of the following outcomes:
  - Irregular cavities, now free of caseation necrosis, may remain as such or **collapse** due to surrounding fibrosis.
  - Involvement of pleura can result in **pleural effusion or tuberculous empyema.**
  - Bacilli may spread to upper respiratory tract via lymphatics or during expectoration of infected material, producing **endobronchial** and **endotracheal tuberculosis.**
  - **Laryngeal** and **intestinal tuberculosis** may follow endotracheal tuberculosis. Tuberculous enteritis spreads via intestinal lymphatics to cause **transverse (circumferential) ulceration**, which may eventually heal by fibrosis to cause **stricture formation.**
  - **Miliary tuberculosis** (Fig. 7.2) is characterized by distinct, yellow-white, firm 1–2 mm (millet like) areas of consolidation that usually do not have grossly visible necrosis or cavitation, but microscopically show typical caseation. It occurs due to lymphatic and haematogenous dissemination from the primary site. Organisms can

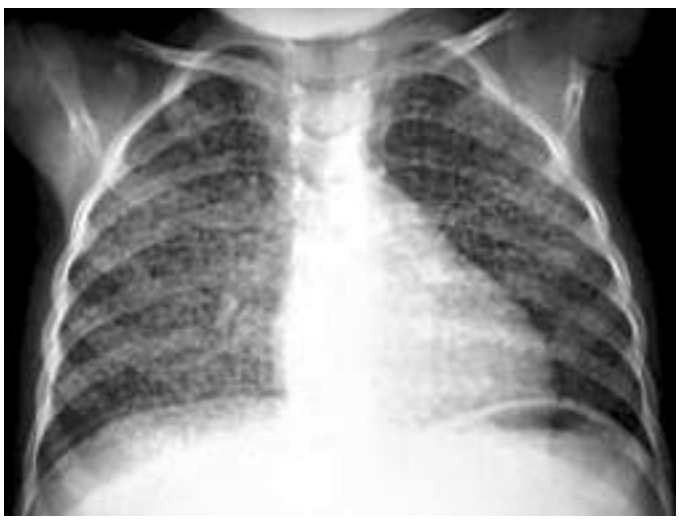


FIGURE 7.2. X-ray lung (PA view) showing millet like areas of consolidation.

drain through lymphatics into lymphatic ducts, which empty into the right side of heart and then into pulmonary arteries. When the dissemination involves only lungs, it is called miliary pulmonary tuberculosis. **Systemic miliary tuberculosis** ensues when infective foci in lungs seed pulmonary venous return to the heart; the organisms subsequently disseminate through systemic arterial system. Almost every organ in body can be seeded.

- **Isolated-organ tuberculosis** is a consequence of haematogenous seeding and organs that are typically involved include **meninges** (tuberculous meningitis), **genital organs** and **urinary tract** (genitourinary tuberculosis), **adrenals** (formerly an important cause of Addison disease) and **bones** (osteomyelitis). When vertebrae are affected, the disease is referred to as **Pott disease**. Paraspinal '**cold abscesses**' in these patients may track along tissue planes to present as an abdominal or pelvic mass. The most common type of extra pulmonary tuberculosis is **lymphadenitis and it most frequently involves the** cervical region. The usual presentation is that of discharging sinuses with an underlying cervical swelling ('**scrofula**'). The lymph nodes are involved as a consequence of lymphatic spread.

Microscopy

Epithelioid cell granulomas with or without caseation are the histological hallmark of tuberculous disease. These granulomas are usually enclosed within a fibroblastic rim. Multinucleate giant cells called 'Langhans giant cells' are present in the granuloma along with mononuclear cells including lymphocytes, plasma cells and histiocytes. Immunocompromised individuals do not form well-defined granulomas and may manifest with ill-formed aggregates of histiocytes and chronic inflammatory cells (see Chapter 2).

The differences between primary and secondary tuberculosis are listed in Table 7.2.

Diagnosis

1. **Demonstration of AFB on microscopic examination of a diagnostic specimen (sputum or tissue):** Smears or tissue slides stained with Ziehl Neelsen stain are examined for AFB. This method has a relatively low sensitivity (40–60%) in confirmed cases of pulmonary tuberculosis. **Auramine-rhodamine staining** and **fluorescence microscopy** can improve the sensitivity to a certain extent. Three sputum specimens, preferably collected early in the morning, should be submitted to the laboratory for AFB smear and mycobacterial culture.
2. **Culture:** Besides sputum and tissue, other specimens which can be used for culture are body cavity fluids, urine or gastric lavage fluid. Specimens may be inoculated onto egg- or agar-based medium (eg, Löwenstein–Jensen or Middlebrook 7H10) and incubated at 37°C. M. *tuberculosis* grows slowly (4–8 weeks). A presumptive diagnosis can be

TABLE 7.2. Differences between primary and secondary tuberculosis

Features	Primary TB	Secondary TB
Evolution of disease	Seen in individuals who have not been previously sensitized to tubercle bacilli	Occurs due to reactivation of a primary focus or reinfection
Age group affected	Common in children/individuals of younger age; may be seen in adults in developed countries	Any age (usually occurs later than primary infection)
Distribution	Lower part of upper lobe and upper part of lower lobe	Apex of one or both lobes due to high oxygen tension in apices
Lesion	Ghon focus	Simon focus
Involvement of lymphatics	Early involvement of lymphatics and lymph nodes	Due to pre-existing hypersensitivity, bacilli induce an immediate tissue response that walls off the lesion and prevents early involvement of lymphatics and lymph nodes
Severity	Generally asymptomatic, less severe	Usually symptomatic, more severe

made based on colony pigmentation and morphology; however, biochemical tests are a must for species recognition.

3. **Molecular typing**: *M. tuberculosis* is isolated and species identification is done by molecular methods or high-pressure liquid chromatography of mycolic acids (reducing the time required for confirmation to 2–3 weeks).
4. **Tuberculin sensitivity test (TST):** It is based on the principle that *M. tuberculosis* in a concentrated liquid culture medium (purified protein extract or PPD) can elicit a skin reaction when injected subcutaneously into patients with tuberculosis. A person is given the tuberculin and asked to return within 48–72 h to have a trained health care worker look for a reaction on the arm (swelling, induration and erythema) and measure its size. Redness by itself is not considered part of the reaction. The lack of mycobacterial species specificity, subjectivity of interpretation and batch-to-batch variations limits the usefulness of PPD.
5. **In vitro assays that measure T cell release of IFN-γ in response to stimulation with the highly tuberculosis-specific antigens ESAT-6 and CFP-10:** These are commercially available assays (Interferon γ release assay or IGRA). IGRAs are more specific than the TST as a result of less cross-reactivity due to BCG vaccination and sensitization by non-tuberculous mycobacteria. IGRAs also appear to be at least as sensitive as the TST for active tuberculosis.

Leprosy

- Also called **Hansen disease**, leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, a weakly acid fast intracellular bacillus.
- **Transmission** occurs by close and prolonged contact with an infected individual or through nasal droplets.
- The incubation period of leprosy can vary from 2 to 10 years.
- It can be experimentally introduced in animals like armadillo and chimpanzee.
- It is possible to grow the bacterium in the laboratory by injection into footpads of mice, however, it does not grow on artificial media or in cell culture.

Pathogenesis

- Genetic factors are thought to play a role in the pathogenesis of leprosy (based on the observation of clustering of leprosy in certain families).
- Malnutrition and prolonged close contact with the infected person facilitates the development of the disease.

Classification

- **Ridley and Jopling** classified leprosy into six categories: indeterminate leprosy, tuberculoid leprosy, borderline tuberculoid leprosy, mid-borderline leprosy, borderline lepromatous leprosy and lepromatous leprosy.
- **World Health Organization (WHO)** has replaced the older, more complicated classification system with a simpler system that identifies two main types of leprosy—*paucibacillary* and *multibacillary*. Paucibacillary leprosy is defined as five or fewer skin lesions with the absence of bacilli in skin smears, and multibacillary leprosy is defined as six or more skin lesions and positive skin smears (Table 7.3).
- ***Paucibacillary leprosy*** (Fig. 7.3) includes indeterminate, tuberculoid and borderline tuberculoid leprosy. It typically presents with one or more hypopigmented skin macules, which show sensory loss (due to peripheral nerve damage caused by the host immune cells).
- ***Multibacillary leprosy*** (Fig. 7.4) includes mid-borderline, borderline lepromatous and lepromatous leprosy. It presents with symmetric skin lesions, nodules, plaques and frequent involvement of nasal mucosa. However, detectable nerve damage is a late occurrence.
- *Borderline leprosy* is a lesion of intermediate severity between pauci and multibacillary leprosy, and is the most common form. Skin lesions resemble tuberculoid leprosy, but are larger, more numerous and irregular; peripheral nerve involvement with loss of sensation is common. This type is unstable and may convert into lepromatous leprosy or may undergo a reversal reaction.

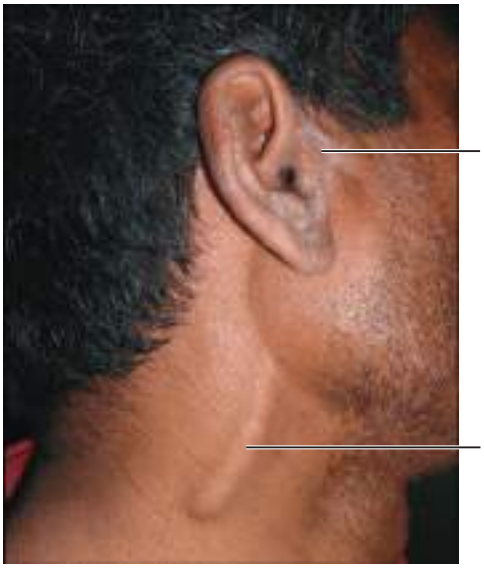


FIGURE 7.3. Large, well-defined, hypopigmented plaque of paucibacillary leprosy showing irregular borders.

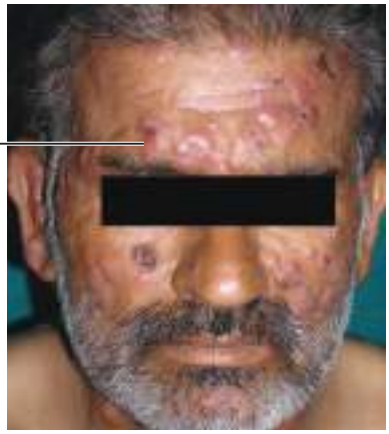


FIGURE 7.4. Multiple, large, variable-sized plaques of lepromatous leprosy.

TABLE 7.3. Differences between tuberculoid and lepromatous leprosy

Features	Tuberculoid leprosy	Lepromatous leprosy
T-cell-mediated immunity	Well developed	Absent/very weak
Lepromin test	Strongly positive	Negative
Skin lesions	Asymmetrical, single or few, well-defined, hypopigmented patches/plaques or erythematous macular lesions; all with sensory loss	Symmetrical multiple, ill-defined, hypopigmented or erythematous, maculopapular or nodular lesions; sensory loss late and less prominent
Histology	Well-formed epithelioid cell granulomas eroding basal layer of epidermis, no clear grenz zone. Paucibacillary	Foamy macrophages/lepra cells in dermis separated from epidermis by a clear grenz zone. Multibacillary
CD4+ T-cells	Present in abundance at periphery of granuloma	Almost absent
CD8+ T-cells	Very few; at centre of lesion	Present (more in number) in a diffuse manner
Infectivity	Low	High
Involvement	Mostly nerve (severely affected, may be destroyed), skin	Skin, peripheral nerves, anterior eye, upper airways, testes, feet, hands
Complications	Related to nerve damage like paralysis, distinct sensory disturbances	Type II immune complex-mediated reaction or erythema nodosum leprosum (ENL) causing vasculitis, glomerulonephritis, nerve-related damage
Prognosis	Milder disease; better prognosis	Extensive, progressive disease; bad prognosis

Clinical Features

- Involvement of nasal passages can result in a chronically stuffy nose; and epistaxis may occur due to mucosal erosion.
- Eye damage can lead to blindness.
- Men with lepromatous leprosy may experience erectile dysfunction (impotence) and become infertile (testicular involvement).
- Deformities may result from muscle weakness.

Reactions in Leprosy

- During the course of treatment (or even in untreated leprosy), a sudden change in the status of host immune response may produce leprosy reactions—an acute inflammatory state. These reactions manifest with fever and inflammation of the skin as well as peripheral nerves, and may affect the lymph nodes, bone marrow, liver, spleen, joints, testes, kidneys and anterior chamber of eye.
- *Type I reaction*: It is a cell-mediated immune reaction (delayed or Type IV hypersensitivity) to mycobacterial antigens in skin and nerves. It may be upgrading or downgrading depending on the predominantly activated cell type, ie, CD4+ T cells or CD8+ T cells, respectively. Patients with borderline disease are usually affected as borderline leprosy is the most unstable form of leprosy. A downgrading reaction represents a shift towards the lepromatous pole, and a reversal reaction represents a shift towards tuberculoid pole.
- *Type II reaction*: Also called erythema nodosum leprosum (ENL), it is a Type III (immune complex mediated) reaction to mycobacterial antigens, usually seen in lepromatous and borderline lepromatous subtypes (clinical variants with antigen excess).

Diagnosis

Diagnosis is confirmed by microscopically examining infected skin tissue (either a slit smear or a skin biopsy).

Q. Write briefly about the aetiology and clinical types of pneumonia.

Ans. Pneumonia is defined as a collection of inflammatory exudate in lung parenchyma distal to terminal bronchioles, mostly resulting in consolidation (solidification) of lung part(s).

Classification

1. **Aetiological**:
   a) *Community-acquired or acute bacterial pneumonia:*
      (i) *Streptococcus pneumoniae* (most common causative organism; typically has lobar distribution)
      (ii) *Hemophilus influenzae and Moraxella catarrhalis* (complicate COPD)
      (iii) *Staphylococcus aureus* (occurs secondary to viral infections)
      (iv) *Legionella pneumophilia (seen in organ transplant patients)*
      (v) *Enterobacteriaceae* (infect chronic alcoholics)
      (vi) *Pseudomonas* (seen in cystic fibrosis and burn patients)
      (vii) *Atypical organisms* (include *Mycoplasma pneumoniae, Chlamydophilia pneumoniae, Coxiella burnetii* and viruses-respiratory syncytial virus, parainfluenza virus, human metapneumovirus, influenza A and B, and adenovirus). They are labelled 'atypical' as they are not demonstrable with Gram-stain and do not grow on routine culture media.
   b) *Healthcare-associated pneumonia*: Distinct clinical entity defined by the following criteria: hospitalization of at least two days within recent past; attending a long-term care facility, a hospital or a haemodialysis clinic; recent intravenous antibiotic therapy, wound care or chemotherapy. Causative organisms include
      (i) *Staphylococcus aureus* (methicillin sensitive)
      (ii) *Staphylococcus aureus* (methicillin resistant)
      (iii) *Pseudomonas* species
      (iv) *Streptococcus pneumoniae*

c) *Hospital-acquired pneumonia*: Defined as pulmonary infections acquired during course of hospital stay. Causative organisms include
   (i) Gram-negative rods
   (ii) *Enterobacteriaceae*
   (iii) *Pseudomonas, Staphylococcus aureus* (methicillin resistant)

d) *Aspiration (inhalation) pneumonia*: Usually seen in debilitated, comatose or unconscious patients. Aspiration of gastric contents results in chemical irritation (due to gastric acid) and also bacterial infection. It is typically associated with anaerobic infection (oral flora) mixed with aerobic organisms.

e) *Chronic pneumonia*: It is a localized lesion with or without lymph node involvement, typically showing granulomatous inflammation. Causative organisms include *Nocardia*, *Actinomyces*, *M. tuberculosis*, atypical mycobacteria, histoplasmosis, *Coccidioides immitis* and *Blastomyces dermatitidis*.

f) *Necrotizing pneumonia* and *lung abscess*: It is caused by anaerobic oral flora mixed with or without aerobic organisms, eg, *Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pyogenes, Pneumococcus* (type III).

g) *Pneumonia in an immunocompromised host*: Caused by opportunistic agents which rarely infect normal hosts, namely, Cytomegalovirus, *Pneumocystis jiroveci, Mycobacterium avium-intracellulare,* invasive aspergillosis and invasive candidiasis. This presents with pulmonary infiltrates with or without other signs.

2. Anatomical distribution (Fig. 7.5):
   - *Lobular/bronchopneumonia*
   - *Lobar pneumonia*

Pathogenesis

Pneumonia usually occurs whenever defence mechanisms of the respiratory system are impaired or immunity of the host is low. The normal respiratory defence mechanisms include

- Nasal clearance (sneezing, blowing)
- Tracheobronchial clearance (mucociliary action)
- Alveolar clearance (alveolar macrophages)

Predisposing Factors

- Loss or suppression of cough reflex as in coma, anaesthesia and after intake of certain drugs
- Injury to mucociliary apparatus/impaired ciliary function, as in cigarette smoking, inhalation of hot or corrosive gases
- Impaired phagocytic or bactericidal action of alveolar macrophages
- Pulmonary congestion and oedema
- Accumulation of secretions, as in bronchial obstruction

Differentiating features of bronchopneumonia and lobar pneumonia are listed in Table 7.4.

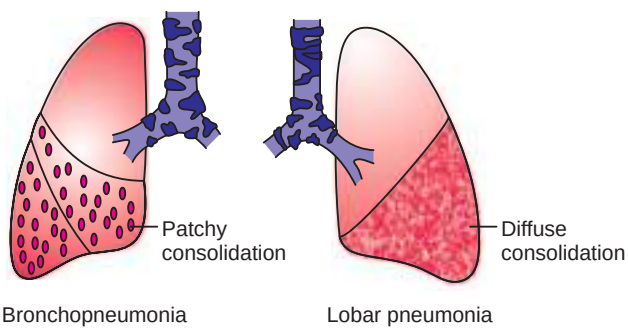


FIGURE 7.5. Anatomical distribution of bronchopneumonia and lobar pneumonia.

TABLE 7.4. Differences between bronchopneumonia and lobar pneumonia

Features	Bronchopneumonia	Lobar pneumonia
Definition	Patchy consolidation of multiple lobes; usually bilateral	Involvement of a large part of a lobe or an entire lobe, diffuse consolidation
Predisposing illness	Bronchitis/bronchiolitis, chronic debility	Affects healthy individuals
Immune status	Usually affects immunosuppressed individuals	Affects previously healthy individuals
Distribution	Basal area more affected as secretions gravitate into lower lobe	May involve any lobe
Stages of inflammation	No clear-cut division	Divided into four stages
Organisms	Staphylococci, Streptococci, Pneumococci, *H. influenzae*, *Pseudomonas aeruginosa*, Coliforms	Pneumococci/*Streptococcus pneumoniae* (95%), Klebsiella, *H. influenzae*
Severity	Less	More
Sputum	Purulent, nonhaemorrhagic	Initially scanty, watery; later thick, purulent, haemorrhagic

Morphological Changes in Lobar Pneumonia

In the era before antibiotics, pneumococcal pneumonia involved entire lobes and was thought to evolve through four stages:

1. **Stage of congestion:** Marked by a prominent acute inflammatory response to bacterial infection.
   Gross
   Affected parts are heavy, boggy and red (congested); cut surface shows blood stained and frothy exudate.
   Microscopy:
   - Dilatation and congestion of vessels in alveolar septae with accumulation of fluid in alveolar spaces
   - Numerous bacteria; few neutrophils and red cells in the alveolar spaces
2. **Stage of red hepatization:**
   Gross
   - Lung is red, firm, consolidated, has a liver-like consistency
   - Cut surface appears airless, red-pink, dry and granular

   Microscopy
   Alveolar spaces are packed with red cells and neutrophils
3. **Stage of grey hepatization:**
   Gross
   Lung is grey in colour; has a dry, granular surface (liver-like consistency)
   Microscopy
   - Lysis of red cells
   - Persistence of fibrinous exudate in the alveoli
   - Reduction in neutrophilic and bacterial numbers, and appearance of macrophages
4. **Resolution:**
   - Exudate within alveolar space undergoes progressive enzymatic digestion to form granular, semifluid debris, which is either coughed up, or reabsorbed and ingested by macrophages.
   - Exudate may undergo organization, resulting in fibrosis or formation of permanent adhesions.

Complications of Pneumonia

- **Abscess formation:** Results from tissue destruction (more in case of Klebsiella or type III Pneumococcal infections)
- **Empyema:** Virulent bacterial strains induce suppuration in the pleural cavity resulting in empyema.

- **Fibrosis:** Organization of intra-alveolar exudate may convert affected lung into solid fibrous tissue.
- **Bacteraemic dissemination:** Dissemination of bacteria may lead to endocarditis, pericarditis, meningitis, suppurative arthritis and formation of metastatic abscesses in various organs, eg, kidneys, spleen, etc.

Primary Atypical Pneumonia (Viral and Mycoplasma Pneumonia/ Interstitial Pneumonitis)

It is defined as an acute febrile respiratory disease which manifests with patchy inflammatory changes confined to alveolar septae and pulmonary interstitium. Causative organisms include

- *Mycoplasma pneumoniae*
- Influenza virus type A and B
- Respiratory syncytial viruses, adenovirus, rhino virus, rubeola and varicella virus
- *Chlamydia*
- *Coxiella burnetii*

Predisposing Conditions

Malnutrition, alcohol intake and diminished immunity

Clinical Features

- Nonspecific
- May mimic upper respiratory tract infection **or** present as an acute nonspecific febrile illness manifesting with fever, headache, myalgias
- May present as a life-threatening infection in immunocompromised individuals

Gross Morphology

- Lungs are red-blue, congested and subcrepitant; pleural involvement is rare.
- Involvement may be patchy or lobar; unilateral or bilateral.

Microscopy

- Inflammation is restricted to alveolar walls and the alveolar space appears free of exudate (therefore, also called atypical pneumonia). Alveolar walls show presence of mononuclear inflammation (lymphocytes, histiocytes and plasma cells).
- Alveolar spaces may sometimes demonstrate intra-alveolar proteinaceous material or a pink hyaline membrane lining the alveolar septal walls.
- Superimposed bacterial infections lead to picture-simulating bacterial pneumonias.
- **Cytomegalovirus-induced atypical pneumonia** is characterized by presence of giant cells with intranuclear/ intracytoplasmic inclusions.

Q. Write briefly about syphilis.

Ans. Though a venereal disease, syphilis involves multiple systems. It is often called 'the great imitator' because many of its signs and symptoms show a major overlap with those of other diseases.

Causative agent is *Treponema pallidum* (Fig. 7.6). The organism has the following characteristics:

- An axial protoplasmic flagella wound around a slender helical protoplasm.
- Confirmation of diagnosis by dark field examination, silver stains and immunofluorescence examination.
- Sexual transmission (through bacteria-laden secretions/intimate contact).
- Transplacental transmission (congenital syphilis).

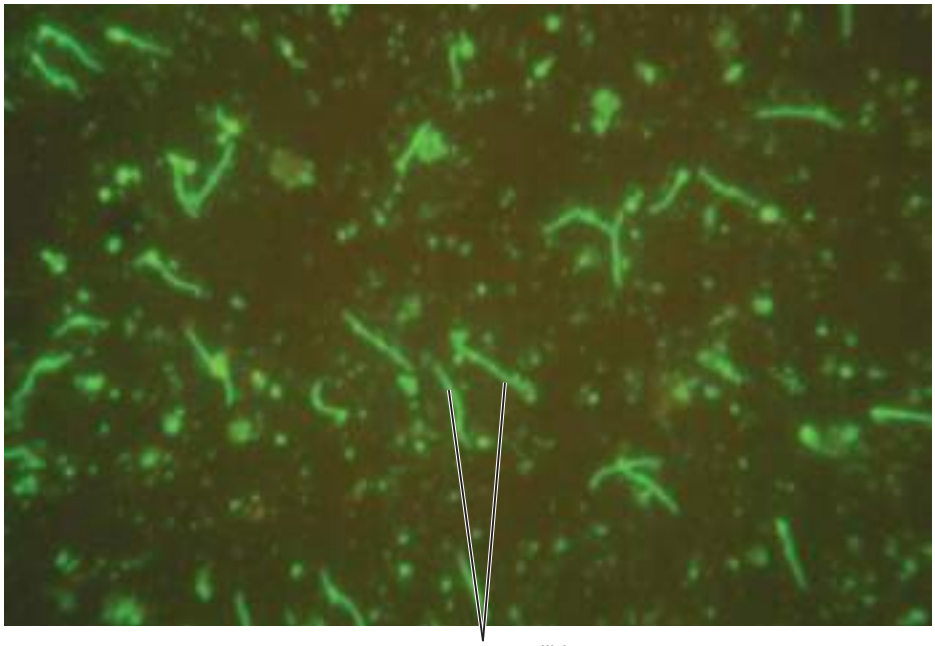


FIGURE 7.6. Demonstration of *Treponema pallidum* on FTA-ABS test.

Clinical Features

1. **Primary stage**
   - The incubation period ranges from 10 to 90 days (average 21 days).
   - The disease starts with a solitary, firm, nontender raised lesion (*chancre;* Fig. 7.7) on penis, cervix, vagina, arms or as multiple sores (*chancres*). The chancre heals in a few days (even without therapy).

FIGURE 7.7. Primary chancre showing a typical clean, well-defined, indurated, nontender ulcer.

2. **Secondary stage**
   - Secondary stage is heralded by appearance of a rash in the skin and mucous membranes, seen as rough, reddish-brown spots, most prominent on palms of hands and soles.
   - The rash may be accompanied by fever, sore throat, lymph node enlargement, patchy hair loss, headache, muscle aches and fatigue.
   - Moist areas of the skin, eg, anogenital region, axillae and inner thighs may develop broad-based, elevated plaques (**condyloma lata**).
   - The signs and symptoms of secondary syphilis may resolve with or without treatment. In the absence of treatment, the infection progresses to latent and tertiary stages of the disease.
3. **Latent stage**
   - The beginning of the latent (hidden) stage of syphilis coincides with the disappearance of the symptoms of the primary and secondary stages.
   - The latent stage can last for years and during this time the infected person continues to harbour syphilis even though there are no active signs or symptoms.
4. **Tertiary stage**
   - About 15% of untreated patients go into late tertiary stage of syphilis.
   - This can happen even 10–20 years after the infection is first acquired and may evolve into neurosyphilis (meningovascular, tabes dorsalis, general paresis), aortitis (aneurysms, aortic regurgitation), gumma formation (hepar lobatum, involvement of skin, bone, etc.) and others.
   - Clinical manifestations include muscle in-coordination, paralysis, numbness, gradual onset of blindness and dementia.
   - Syphilis affecting pregnant women may lead to late abortion or stillbirth.
   - Infantile syphilis may manifest with the rash, osteochondritis, periostitis, liver and lung fibrosis.
   - Childhood syphilis usually manifests with interstitial keratitis, Hutchinson's teeth and eighth nerve degeneration.

Histological hallmarks of syphilis include

- Obliterative endarteritis (which is seen in H&E sections as endothelial proliferation and obliteration of the lumina by plump endothelial cells). Endarteritis is secondary to binding of spirochetes to endothelial cells through host fibronectin molecules, and is mediated by delayed hypersensitivity reaction.
- Also seen is a mononuclear infiltrate rich in plasma cells.

Diagnostic Tests for Syphilis

These can be classified into

1. **Non-treponemal antibody tests**
   - VDRL (Venereal Disease Research Laboratory) test
   - RPR (Rapid Plasma Regain) test

   These tests detect and quantify the antibody to cardiolipin (a phospholipid common to both host tissues and *T. pallidum*). They become positive only 4–6 weeks after the infection (making immunofluorescence of exudates from the chancre an important investigation early in the course of the disease). They are used as screening tests and for monitoring response to treatment because they become negative after therapy.
2. **Treponemal antibody tests**
   - FTA–ABS (fluorescent treponemal antibody absorption) test
   - Microhaemagglutination assay for *T. pallidum* antibodies

   These tests measure antibodies that specifically react with *T. pallidum*, also become positive 4–6 weeks after the infection, and remain positive even after successful treatment. They are better than non-treponemal antibody tests in terms of specificity, but are not recommended as primary screening tests because they are not cost-effective. They cannot be used to monitor therapeutic response because they continue to be positive even after successful treatment.

Q. Write briefly about the various types of viral diseases.

Ans. Common viral diseases include

Measles

Pathogenesis

- Measles is usually seen in unvaccinated individuals or in cases of vaccination failure. It is caused by an ssRNA virus of the paramyxovirus family (Rubeola virus) and spreads by respiratory droplets.
- The virus initially multiplies within upper respiratory epithelial cells, and then spreads to lymphoid tissues, where it can replicate in mononuclear cells, including T lymphocytes, macrophages and dendritic cells.
- Attachment to respiratory epithelial cells is via receptors, mainly, CD 46 (a complement regulatory protein) and SLAM (signalling lymphocyte activation molecule). The virus then spreads by blood throughout the body.

Clinical Manifestations

- The incubation period of measles (from exposure to onset of rash) is generally 14 days (range 7–18 days). Patients are usually contagious from 4 days before until 4 days after onset of the rash.
- Patient presents with fever, cough, coryza (running nose), conjunctivitis and an erythematous maculopapular rash (a hypersensitivity reaction to viral antigens in the skin).
- **Koplik spots**—a rash (enanthem) present on mucous membranes—is considered pathognomonic of measles. This is typically seen as clustered, white lesions on the buccal mucosa near each Stenson's duct (opposite to the maxillary 2nd molars). On microscopy, Koplik spots appear as ulcerated mucosal lesions with neutrophilic exudate.

Complications

- Diarrhoea
- Middle-ear infection
- Keratitis
- Pneumonia
- Encephalitis, frequently resulting in permanent brain damage (subacute sclerosing panencephalitis or SSPE).

Histopathology

- Skin vessels are dilated and surrounded by a mononuclear perivascular infiltrate.
- Lymphoid organs show follicular hyperplasia.
- Random giant cells called ***Warthin–Finkeldey cells*** may be observed.
- Eosinophilic intranuclear and intracytoplasmic inclusions may be seen in the mononuclear epithelial and giant cells.

Investigations

- Measles serology
- Viral culture (rarely done)

Mumps

Pathogenesis

- It is a transient inflammation of salivary glands caused by an RNA virus that usually also involves testes, pancreas and CNS (causes aseptic meningitis and encephalitis).
- It spreads by respiratory droplets to enter respiratory epithelium, salivary gland tissue and T cells in lymph nodes.
- It can then spread to other sites, including CNS, testis and ovary and pancreas. Aseptic meningitis is the most common extrasalivary complication of mumps infection. Others include orchitis leading to sterility, pancreatitis and encephalitis.

Histopathology

1. **Mumps parotitis**
   - Involvement is bilateral in 70% cases; affected glands are enlarged, congested and inflamed.
   - Interstitium is oedematous and shows infiltration by histiocytes and lymphocytes, which may damage the acini. Ductal lumina may show necrotic debris.
2. **Mumps orchitis**
   - Haemorrhage and infarction may be followed by scarring leading to sterility.
   - Microscopy shows mononuclear cell infiltration.
3. **Mumps pancreatitis**
   - Lesions may be destructive and result in parenchymal and fat necrosis.
   - Neutrophil-rich inflammation is invariably present.
4. **CNS**
   Demyelination and perivascular cuffing may be seen.

Infectious Mononucleosis

Pathogenesis

- Also known as 'kissing disease' or 'Pfeiffer disease' or 'glandular fever', it is a benign, self-limiting, lymphoproliferative disease caused by Epstein–Barr virus (EBV).
- EBV infects B lymphocytes to induce reactive lymphocytosis with presence of atypical lymphocytes known as ***Downey bodies***.
- It is typically transmitted from asymptomatic individuals through close contact and oropharyngeal secretions (earning it the name 'the kissing disease') or by sharing utensils. It may also be transmitted through blood.
- The virus binds to CD21 on the surface of B cells in oropharynx.
- Circulating B cells then spread the infection throughout reticuloendothelial system, ie, liver, spleen and peripheral lymph nodes.
- EBV infection of B lymphocytes results in a humoral and cellular response to the virus. (The humoral immune response directed against EBV structural proteins is the basis for the test used to diagnose infectious mononucleosis.) The T lymphocyte response is essential for the control of EBV infection; natural killer (NK) cells and CD8+ cytotoxic T cells control proliferating B lymphocytes infected with EBV.

Clinical Features

Most commonly affects adolescents and young adults, and is characterized by lymphadenopathy, fever, sore throat, muscle soreness and fatigue. Other manifestations include

- Massive splenomegaly with hepatomegaly
- Petechial haemorrhages and skin rash
- Headache and loss of appetite
- Dizziness or disorientation

Complications

- Hepatitis
- Meningitis and encephalitis
- Pneumonitis
- Rupture of spleen
- EBV is also implicated in the genesis of malignancies like nasopharyngeal carcinoma, Burkitt lymphoma and B cell variety of non-Hodgkin lymphoma.

Diagnosis

- **Peripheral blood**
  - Absolute lymphocytosis
  - Numerous large atypical lymphocytes with abundant basophilic cytoplasm showing vacuolation with an oval, indented, folded nucleus.
- **Lymph nodes**
  - Atypical lymphocytes in paracortical region
  - Enlarged lymphoid follicles with infiltration by atypical lymphocytes

- **Liver**
  - Atypical lymphocytes in portal areas and sinusoids
  - Scattered isolated/individual cell necrosis or foci of parenchymal necrosis common
- **CNS**
  - Congestion, oedema, perivascular mononuclear cells and leptomeningeal infiltrate
- **Mononucleosis test**
  - Includes the **Monospot** test and **EBV antibody** test (Monospot test is a heterophile antibody test for rapid diagnosis of
  - EBV; the test may be falsely negative early in the course of the infection)

Poliomyelitis

Pathogenesis

- Polio is an acute infection of both the meninges and motor neurons of spinal cord and brainstem. Involvement of the latter may produce permanent paralysis.
- It is caused by poliovirus, which is a spherical, unencapsulated RNA virus of the enterovirus genus. There are three major strains of poliovirus: types I, II and III.
- Poliovirus, like other enteroviruses, is transmitted by the faecal-oral route. It first infects tissues in the oropharynx where it infects cells by binding to CD155, is secreted into saliva and swallowed, and then multiplies in intestinal mucosa and lymph nodes, causing a transient viraemia and fever.
- Virus spread to the nervous system may be secondary to viraemia or by retrograde transport of the virus along axons of motor neurons. Circulating viruses cross the blood–brain barrier and cause inflammation (itis) of the grey matter (polio) of the spinal cord (myelin). Motor neurons are involved. In fatal cases, destruction is found in cerebral ganglia, reticular formation, cerebellar nuclei, hypothalamus, thalamus and cerebral cortex.

Clinical Features

- Nonspecific symptoms, eg, moderate fever, headache, vomiting, constipation, coryza and sore throat occur 6–20 days after exposure.
- The illness may subside entirely (minor or abortive poliomyelitis), abate temporarily or progress directly to involve the CNS (major poliomyelitis) 2–6 days after onset, which may be paralytic or nonparalytic.
- Early in paralytic poliomyelitis the patient exhibits
  - Signs of meningeal irritation
  - Weakness
  - Hyperesthesia (increased sensitivity to stimuli)
  - Severe muscle pain
  - Spasm of involved muscles or accentuated tendon reflexes

Pathology

Gross: Swelling, softening, congestion and petechial haemorrhages in the organ affected
Microscopy: Congestion, interstitial oedema and infiltration by lymphocytes

German Measles (Rubella)

Pathogenesis

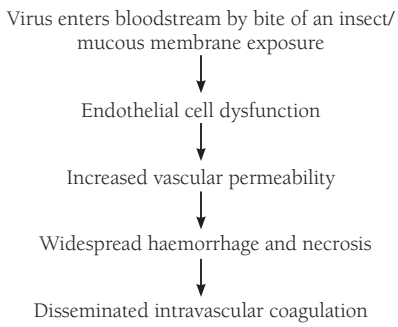
- Also called '3-day measles', it is transmitted by close personal contact and usually presents with fever, headache, arthralgias and painful post-auricular lymphadenopathy.
- This is followed by the onset of a maculopapular rash, which begins on head and spreads downwards.
- In pregnant women, it can cause
  - Cardiac anomalies, such as, ventricular septal defect and patent ductus arteriosus
  - Cataract
  - Deafness
  - Mental retardation and delayed milestones
  - Seizures
  - Microcephaly
  - Intrauterine death

Parvovirus B19

Produces erythema infectiosum (fifth disease), which typically manifests with a confluent maculopapular rash, usually beginning on the cheeks (giving a 'slapped-face' appearance) and extending centripetally to involve trunk.

Viral Haemorrhagic Fevers

- These are systemic infections characterized by fever and haemorrhages. They are caused by enveloped RNA viruses belonging to four different families: arena virus, filo virus, bunya virus and flavivirus.
- The viruses survive in animals and are transmitted by insects. Humans are infected when they come in contact with infected hosts and vectors.
- Manifestations vary from a mild illness (fever, myalgias, headache, rash, neutropenia and thrombocytopenia) to life-threatening disease (haemodynamic disturbances and shock; Flowchart 7.3).



FLOWCHART 7.3. Pathogenesis of viral haemorrhagic diseases.

Herpes Virus

Herpes viruses are large encapsulated viruses having a double-stranded DNA genome. They cause acute infection followed by latent infection in which the viruses persist in a noninfectious form with periodic reactivation. There are nine types of human herpes viruses, belonging to three subgroups defined by the type of cell most frequently infected and site of latency (Table 7.5):

- **α-Group viruses:** Herpes simplex virus-1 (HSV-1), HSV-2 and varicella zoster virus (VZV)
- **β-Lymphotropic viruses:** CMV, human herpes virus-6 (which causes exanthem subitum, also known as roseola infantum and sixth disease—a benign rash of infants) and human herpes virus-7 (which is not yet associated with a specific disease)
- **γ-Group viruses:** EBV and KSHV/HHV-8 (Table 7.5)—the cause of Kaposi sarcoma

Herpes Simplex Infection

- Causative organisms are HSV-1 and HSV-2, both of which cause primary and secondary as well as acute and latent infections.
- Both viruses replicate in the skin and mucous membranes at the *site of entry (oropharynx and genitals)* and produce infective virions which induce vesicular lesions. The virions then spread to sensory neurons that innervate the primary sites.
- Virus is transported along axons to neuronal cell bodies where the virus establishes latent infection and is not immunologically recognized. In an immunocompetent individual, infection resolves in a few days, but the virus remains dormant in nerve cells. Reactivation occurs repeatedly when the virus travels from neurons to skin and mucous membranes.

TABLE 7.5. Types of herpes viruses

Herpes type	Name	Target cell	Site of latency	Transmission
1	HSV-1	Epithelial cells	Neurons	Close contact
2	HSV-2	Epithelial cells	Neurons	Close contact usually sexual
3	Varicella Zoster virus (VSV)	Epithelial cells	Neurons	Contact or respiratory route
4	Epstein–Barr virus (EBV)	B lymphocytes, epithelial cells	B lymphocytes	Saliva
5	Cytomegalovirus (CMV)	Epithelial cells, monocytes, and lymphocytes	Monocytes and lymphocytes	Contact, blood transfusions, transplantation, congenital
6	Herpes lymphotropic virus	T lymphocytes	T lymphocytes	Contact, respiratory route
7	Human herpes virus-7 (HHV-7)	T lymphocytes	T lymphocytes	Unknown
8	Human herpes virus-8 (HHV-8)/ Kaposi sarcoma-associated herpes virus (KSHV)	Endothelial cells	Unknown	Possibly exchange of body fluids

Clinical Manifestations

- **Oral herpes** can be caused by HSV-1 or HSV-2. In **primary herpetic gingivostomatitis**, the typical clear lesions are the first to develop followed by ulcers. The infection starts on the lips and spreads to all parts of the mouth and pharynx.
- Reactivation from the trigeminal ganglia can result in what are known as **cold sores**. Intraepithelial vesicles (due to intracellular oedema and ballooning of cells) are formed, which burst and crust, and can lead to superficial ulceration.
- Swollen, erythematous HSV lesions of fingers or palm (**herpetic whitlow**) occur in infants and occasionally, in healthcare workers.
- **Herpes keratitis** is an infection of the eye primarily caused by HSV-1. It can be recurrent and may lead to blindness.
- **Genital herpes** is usually the result of HSV-2 with about 10% of cases being the result of HSV-1. Primary infection is often asymptomatic, but sometimes painful lesions can develop on glans or shaft of the penis in men and on vulva, vagina, cervix and perianal region of women. HSV-2 can be transmitted to neonates during passage through the birth canal of infected mothers. HSV-2 disease in the neonate can vary from being mild to severe with generalized lymphadenopathy, splenomegaly and necrotic foci throughout the lungs, liver, adrenals and central nervous system.
- **Eczema herpeticum** is characterized by confluent, pustular or haemorrhagic blisters, often with bacterial superinfection and viral dissemination to internal viscera.
- **Herpes bronchopneumonia** can result from insertion of an airway through oral herpes lesions.
- **Herpes hepatitis** can cause liver failure.
- **HSV** can be a cause of inflammation of rectum and anus (proctitis).
- **HSV encephalitis** due to HSV-1 infection is the most common sporadic viral encephalitis.
- **HSV meningitis** is the result of HSV-2 infection and usually resolves spontaneously.

Morphology

- Morphologic hallmark of HSV infection is *large pink-to-purple intranuclear (Cowdry type A) inclusions,* which push nuclear chromatin to periphery.
- There is a mild increase in cellular size along with the formation of multinucleated syncytial cells that also have inclusions (Fig. 7.8).

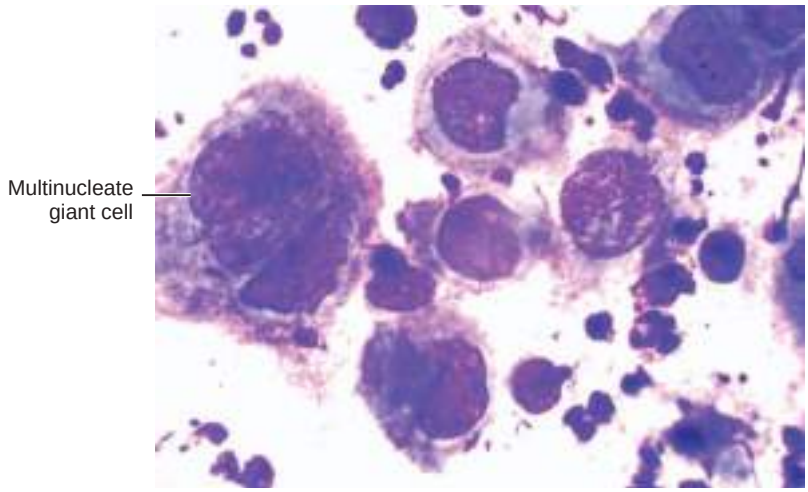


FIGURE 7.8. Herpes-infected enlarged keratinocytes with multinucleated syncytial cells.

Cytomegalovirus (CMV)

Pathogenesis

- CMV can produce a variety of disease manifestations, depending on the age of the host, and, more important, on the host's immune status.
- The major glycoprotein envelope of CMV binds to epidermal growth factor receptor (EGFR) to gain entry into different cells.
- CMV infects and remains latent in white blood cells, and can be reactivated in the event of depressed cell-mediated immunity (CMI). In immunocompromised patients, CMV can cause life-threatening illness.

Morphology

CMV produces cellular and nuclear enlargement true to its name. A large intranuclear inclusion surrounded by a clear halo (owl's eye) is its morphological hallmark.

Dengue Fever

Pathogenesis

- Dengue ('break-bone') fever is an infectious disease common in tropics. It occurs in epidemic form from time-to-time.
- Dengue is transmitted by several species of mosquitoes within the genus *Aedes*, principally *A. aegypti*.
- Dengue fever virus (DENV) is an RNA virus of family *Flaviviridae*; genus *Flavivirus*.
- The virus has four different types; infection with one type usually gives life-long immunity to that type, but only short-term immunity to others. Subsequent infection with a different type increases the risk of severe complications.

Clinical Features

- The World Health Organization's 2009 classification divides dengue fever into two groups: uncomplicated and severe.
- Most people infected with dengue virus are asymptomatic or only have mild symptoms such as an uncomplicated fever. Others present with a more severe illness, which in a small proportion of cases may be life threatening.
- The incubation period ranges from 3 to 14 days, but most often is about 4–7 days.
- The characteristic symptoms of dengue are sudden-onset fever, headache (typically located behind the eyes), muscle and joint pains and rash. The course of infection is divided into three phases: **febrile, critical** and **recovery.** The **febrile phase** involves high fever, often over 40°C (104°F), and severe generalized aches and pains; this usually lasts 2–7 days. This is followed by a maculopapular rash, after which the disease proceeds to

the **critical phase**, which is marked by resolution of the high fever. During this phase, there may be significant fluid accumulation in the chest and abdominal cavity due to increased capillary permeability and leakage. The **recovery phase** occurs next, with resorption of the leaked fluid into the bloodstream. This is characterized by severe itching and bradycardia, and leads to depletion of fluid from the circulation and decreased blood supply to vital organs. Another rash may occur with either a maculopapular or vasculitic appearance, which is followed by peeling of the skin.

- In a small proportion of cases, the disease develops into a life-threatening **dengue haemorrhagic fever**, resulting in bleeding, low circulating levels of platelets and blood plasma leakage, or into **dengue shock syndrome**, where dangerously low blood pressure occurs. Polymorphisms in particular genes have been linked with increased risk of severe dengue complications.

Q. Write briefly about chlamydial infections.

Ans. *Chlamydia trachomatis is* a small Gram-negative, aerobic, intracellular bacterium.

- *Chlamydia pneumoniae* is one of the main causative agents of pneumonia and bronchitis. It has also been linked with atherosclerosis and multiple sclerosis.
- C. *trachomatis* infection causes urogenital infections (*nongonococcal urethritis or* NGU), inclusion conjunctivitis, lymphogranuloma venereum, epididymitis, prostatitis, pelvic inflammatory disease (PID), pharyngitis, conjunctivitis and trachoma.
- Chlamydia exists in two forms during its unique life cycle. The infectious form—the elementary body (EB)—is a metabolically inactive, spore-like structure, which is taken up by host cells, primarily by receptor-mediated endocytosis. The bacteria prevent fusion of endosome and lysosome. Inside the endosome, the EB differentiates into a metabolically active form called the reticulate body (RB) that is capable of infecting additional cells.
- C. *trachomatis* urethritis is characterized by a mucopurulent discharge which on microscopy shows mainly neutrophils. The lesions of lymphogranuloma venereum show a suppurative (neutrophilic inflammatory) response with an occasional granuloma. Intracytoplasmic Chlamydial inclusions can be demonstrated in epithelial or inflammatory cells.
- Regional lymphadenopathy is common. Affected nodes show a granulomatous reaction associated with irregular necrosis (stellate abscesses), which may heal with extensive fibrosis to cause lymphatic obstruction with lymphoedema.
- Chlamydiae cannot be demonstrated by Gram's staining. While culturing of the organism can confirm the diagnosis, this method is limited to research laboratories. For routine diagnostic use, newer and inexpensive diagnostic tests that depend on identification and amplification of the genetic material of the organism have replaced the older, time-consuming culture methods.

Q. Write briefly about rickettsial infections.

Ans. *Rickettsial organisms* are vector-borne, Gram-negative, obligate intracellular bacteria that are divided into two antigenically defined groups: spotted fever group and typhus group.

- Patients present with fever and exanthem; eventually there is visceral involvement; symptoms include nausea, vomiting, abdominal pain, encephalitis, hypotension, acute renal failure, respiratory distress and coma.
- The organisms proliferate in the endothelial cell cytoplasm and then either burst the cell (typhus group) or spread from cell-to-cell (spotted fever group).

Q. Write briefly about fungal infections.

Ans. Fungi are eukaryotes that possess thick chitin-containing cell walls and ergosterol-containing cell membranes. They can grow either as budding yeast cells or as slender filamentous hyphae. Hyphae may be septate (with cell walls separating individual cells) or aseptate, which is an important distinguishing characteristic in clinical material. Fungi may cause superficial or deep infections.

- **Superficial fungal infections** involve the skin, hair and nails. Fungal species that are confined to superficial layers of the human skin are known as dermatophytes. These

infections are commonly referred to by the term 'tinea' followed by area of the body affected (eg, tinea pedis: 'athlete's foot'; tinea capitis: 'ringworm of the scalp'). Certain fungal species invade the subcutaneous tissue, causing abscesses or granulomas, (eg, sporotrichosis and tropical mycoses).

- **Deep fungal infections** can spread systemically and invade tissues, destroying vital organs in immunocompromised hosts, but usually heal or remain latent in otherwise normal hosts, eg, *Histoplasma,* opportunistic fungi like *Candida, Aspergillus, Mucor, Cryptococcus* and *Pneumocystis jiroveci (carinii).*

Candida

- It is a part of normal flora (commensal) of the skin, mouth, gastrointestinal tract and vagina, and does not usually produce any disease. However, some *Candida* species, most often *C. albicans,* can cause human fungal infections, particularly in immunocompromised persons (diabetics, AIDS patients, burn patients, patients receiving transplants and those with haematolymphoid malignancies).
- *Candida* can be directly introduced into the blood by intravenous lines and catheters, during peritoneal dialysis, cardiac surgery or intravenous drug abuse.

Pathogenesis

- *Candida* can shift between different phenotypes in a reversible manner. Phenotypic switching provides a way for *Candida* to adapt to changes in host environment.
- They produce *adhesins* that aid in its adherence to host cells, and *enzymes that* contribute to invasiveness, such as proteinase (degrades extracellular matrix proteins) and catalase (resists oxidative killing by phagocytic cells).
- *Candida* also secretes adenosine, which blocks neutrophil oxygen radical production and degranulation.

Clinical Manifestations

- Candida can cause superficial to disseminated deep mycosis (vaginitis; oral thrush; diaper rash; endocarditis; meningitis; osteomyelitis; and renal, intracerebral and hepatic abscesses).
- In immunocompetent persons, candidiasis is usually a localized infection of the skin or mucosal membranes. Most common type of superficial candidiasis is infection of oral mucosa **(thrush),** which is characterized by formation of a dirty-looking pseudomembrane composed of colonies of organisms and inflammatory debris. Other forms of oropharyngeal candidiasis include thrush, glossitis, stomatitis and angular cheilitis (perleche). **Candida esophagitis** presents with dysphagia, and endoscopy demonstrates white plaques (pseudomembranes) on oesophageal mucosa.
- **Mucocutaneous candidiasis** includes intertrigo, diaper candidiasis, paronychia and onychomycosis.
- **Candida vaginitis** is a common form of vaginal infection in women; especially, those who are diabetic or pregnant or on oral contraceptive pills. It is usually associated with intense itching and a thick, curd-like discharge.
- **Severe disseminated candidiasis** is associated with severe immunosuppression. Candidal sepsis can eventually cause shock and DIC.

Morphology

Candida exists as a yeast form (small, thin-walled ovoid cells of 4–6 microns that reproduce by budding) as well as pseudohyphae, which are best demonstrated by Silver Methenamine and PAS stains (Fig. 7.9). Pseudohyphae are important diagnostic clue for *C. albicans* and represent budding yeast cells joined end-to-end at constrictions, thus simulating true fungal hyphae. Three types of histopathological reactions may be seen:

- No cellular response
- Suppurative response
- Granulomatous response

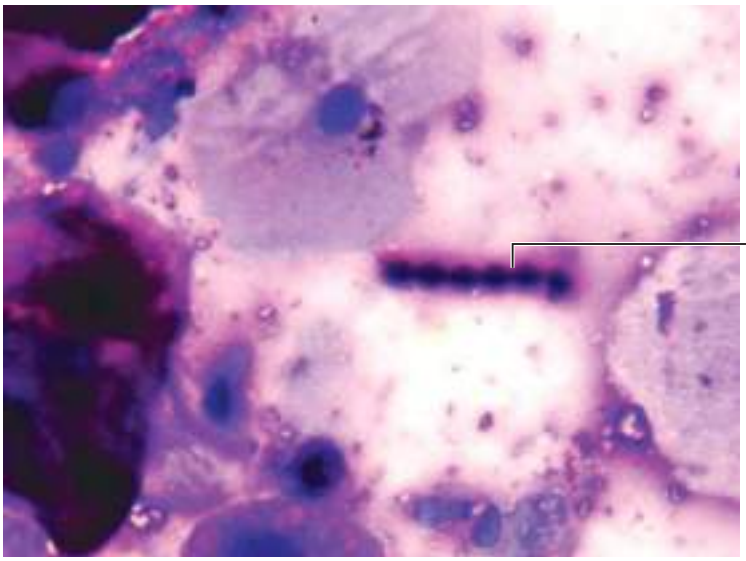


FIGURE 7.9. Yeast forms of Candida (small, thin-walled ovoid cells of 4–6 microns that reproduce by budding and form pseudohyphae; PAS stain; 400×).

Cryptococcosis

- Cryptococcosis is a rare fungal infection caused by inhalation of *Cryptococcus neoformans,* an encapsulated fungus that is ordinarily found in soil.
- Once inhaled, the infection may heal on its own, remain localized in the lungs, or spread throughout the body (dissemination).
- Cryptococcosis mostly occurs in immunocompromised individuals. In people with normal immune system, the infection may have no symptoms. However, in people with impaired immune systems, *Cryptococcus* may even spread to the brain (causing meningoencephalitis). Disseminated cryptococcosis usually involves the skin, liver, spleen, adrenals and bones.

Pathogenesis

Virulence is due to capsular polysaccharides and enzymes, which prevent phagocytosis by alveolar macrophages and inhibits leukocyte recruitment and migration.

Morphology

- Cryptococcus has yeast but no hyphal forms. It is 5–10 microns in size and has a thick gelatinous capsule that is valuable for diagnosis (Fig. 7.10).
- Capsular polysaccharide stains intense red with periodic acid-Schiff (PAS) and mucicarmine stains in tissues, and can be detected with antibody-coated beads in an agglutination assay. India ink preparation gives a negative image, visualizing the thick capsule as a clear halo, but not staining the yeast form.
- In immunosuppressed patients, organisms may evoke virtually no inflammatory reaction, so gelatinous masses of fungi are seen in the tissue (gelatinous reaction). In nonimmunosuppressed patients, the fungi induce a chronic granulomatous reaction. Suppuration is rare.

Molds

Aspergillosis

This saprophytic fungus sporulates and produces conidia (asexual spores) that are readily aerosolized. Molecular studies of *Aspergillus* isolated from opportunistic infections show many different strains of *Aspergillus, Aspergillus fumigatus* is the most common species to cause disease.

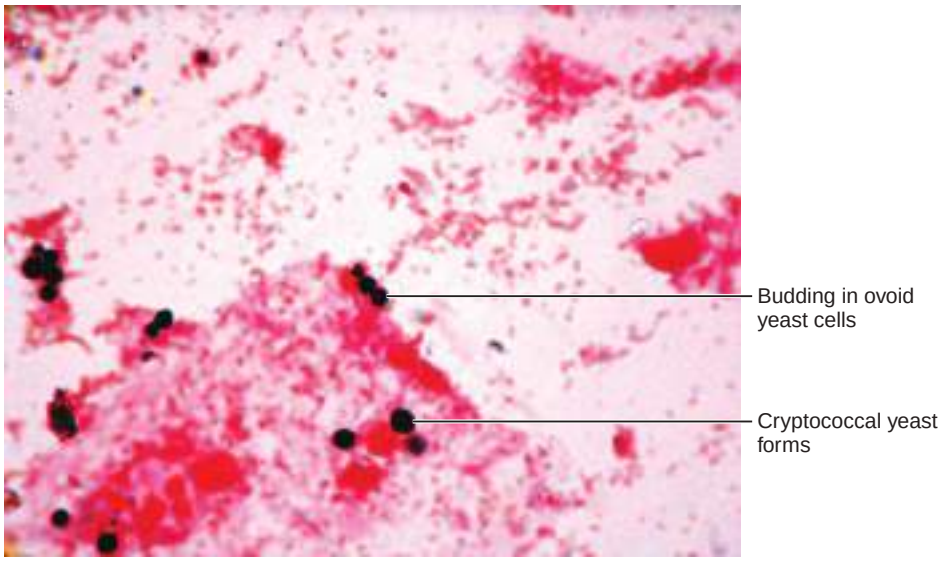


FIGURE 7.10. Yeast forms of *Cryptococcus neoformans* showing a lot of size variation.

Pathogenesis

- The small size of *Aspergillus* spores enables them to reach alveoli where they are taken up by alveolar macrophages, which secrete cytokines and chemokines to elicit adaptive immune responses.
- *Aspergillus* produces several virulence factors, including adhesins, antioxidants, enzymes and toxins. *Aspergillus* species is a source of aflatoxin, which is a major cause of liver cancer in Africa. Sensitization to *Aspergillus* spores can produce an allergic alveolitis.
- **Allergic bronchopulmonary aspergillosis** results from hypersensitivity arising from superficial colonization of bronchial mucosa and may eventually result in chronic obstructive lung disease.
- **Colonizing aspergillosis (aspergilloma)** is defined as growth of the fungus in pulmonary cavities with minimal or no invasion of the tissues. Cavities usually result from pre-existing tuberculosis, bronchiectasis, old infarcts or abscesses. Masses of fungal hyphae called fungus balls are seen lying free within the cavities. They may be surrounded by minimal inflammatory reaction to marked chronic inflammation and fibrosis.
- **Invasive aspergillosis** is an opportunistic infection that is confined to immunosuppressed and debilitated hosts.

Morphology

- *Aspergillus* forms fruiting bodies (particularly in cavities) and septate filaments, which are 5–10 microns thick and branch at acute angles (Fig. 7.11).
- It has a tendency to invade blood vessels; therefore, areas of haemorrhage and infarction are usually superimposed on necrotizing, inflammatory tissue reactions.
- In invasive aspergillosis, the primary lesions are usually in the lung, but widespread haematogenous dissemination is common. The pulmonary lesions take form of necrotizing pneumonia with sharply delineated, rounded, grey foci with haemorrhagic borders, often referred to as **target lesions.**

Zygomycosis (Mucormycosis)

- Zygomycetes form nonseptate, broad (6–50 microns) fungal hyphae with frequent right-angled branching, which are readily demonstrated in the necrotic tissues by haematoxylin and eosin or special fungal stains.
- Also called mucormycosis or phycomycosis, zygomycosis is an opportunistic infection caused by 'bread mold fungi', including *Rhizopus, Absidia, Cumunghanrella* and *Mucor,* which belong to the class Zygomycetes.

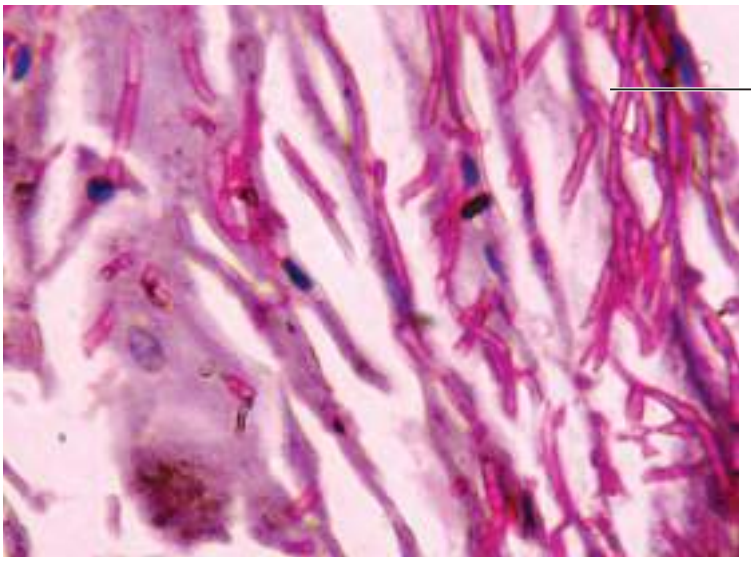


FIGURE 7.11. Septate filaments of *Aspergillus* showing branching at acute angles (PAS; 200×).

- The three primary sites of invasion are nasal sinuses, lungs and gastrointestinal tract, depending on whether spores (which are widespread in dust and air) are inhaled or ingested.
- Most commonly in diabetics, the fungus may spread from nasal sinuses to orbit and brain, giving rise to **rhinocerebral mucormycosis.** The zygomycetes cause local tissue necrosis, invade arterial walls and penetrate periorbital tissues and cranial vault. Meningoencephalitis follows, sometimes complicated by cerebral infarctions when fungi invade arteries and induce thrombosis.
- **Lung involvement** with zygomycetes (Fig. 7.12) may be secondary to rhinocerebral disease, or it may be primary in patients with haematologic neoplasms. Lung lesions are a combination of haemorrhagic pneumonia with vascular thrombi and infarcts.

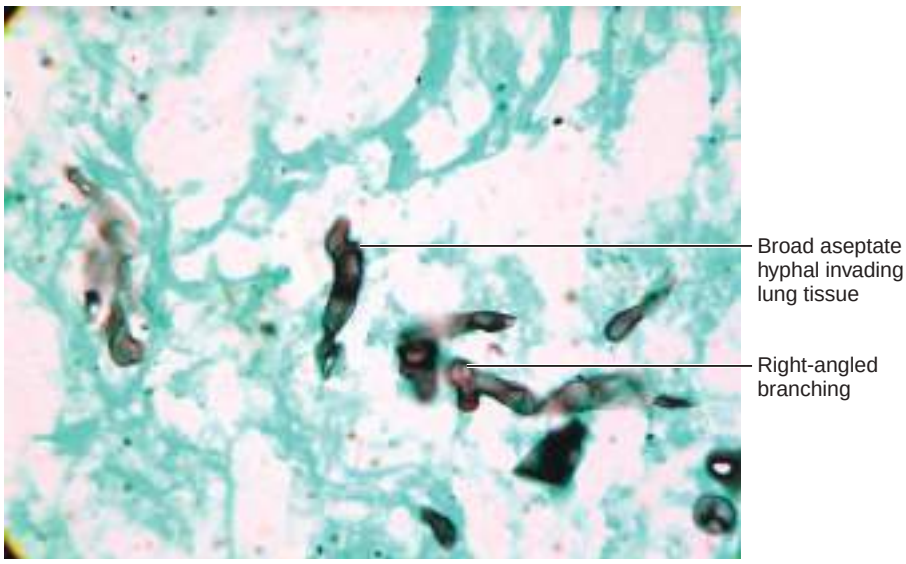


FIGURE 7.12. Lung parenchyma showing invasion by broad aseptate hyphae of zygomycetes branching at right angle (Silver stain′ 400×).

Mycetoma

Mycetoma can be classified as a **Eumycetoma—**a fungal disease, or **Actinomycetoma**—an old name for Actinomycosis.

Eumycetoma

- It is a chronic, specific, granulomatous, fungal disease which mainly affects the foot. *Mycetoma pedis* is also known as **Madura foot (**7.13**a)**. This infection is endemic in Africa, India and Central and South America and is usually acquired while performing agricultural work due to contact with grains of fungal spores that have been discharged onto soil.
- Infection usually manifests as an open area or break in the skin. It is clinically characterized by draining sinuses, granules and tumefaction.
- The disease usually involves cutaneous and subcutaneous tissue, and may also spread to underlying fascia and bone. Sinuses discharge serosanguinous fluid containing granules that vary in size, colour and degree of hardness, depending on aetiologic species, and are hallmark of mycetoma (Fig. 7.13).
- Eumycetoma may be of several varieties, depending on colour of granulous discharge:
  - Red—*Actinomadura pelletieri*
  - White or yellow—*Acremonium species, Aspergillus nidulans, Pseudallescheria boydii*
  - Black—*Curvularia lunata, Exophiala jeanselmei, Madurella grisea, Madurella mycetomatis*

Actinomycetoma

- Actinomycosis is a rare, chronic and slowly progressive granulomatous disease caused by filamentous, Gram-positive, anaerobic bacteria from the *Actinomycetaceae* family (genus *Actinomyces*) such as *Actinomyces israelii* or *A. gerencseriae*. It can also be caused by *Propionibacterium propionicus*.
- Actinomyces are commensals of the human oropharynx, gastrointestinal tract and urogenital tract. When tissue integrity is breached through a mucosal lesion they can invade local structures and organs and become pathogenic.
- The condition tends to affect certain areas of the body and can be classified into four main types:
  - Oral cervicofacial actinomycosis
  - Thoracic actinomycosis
  - Abdominal actinomycosis
  - Pelvic actinomycosis

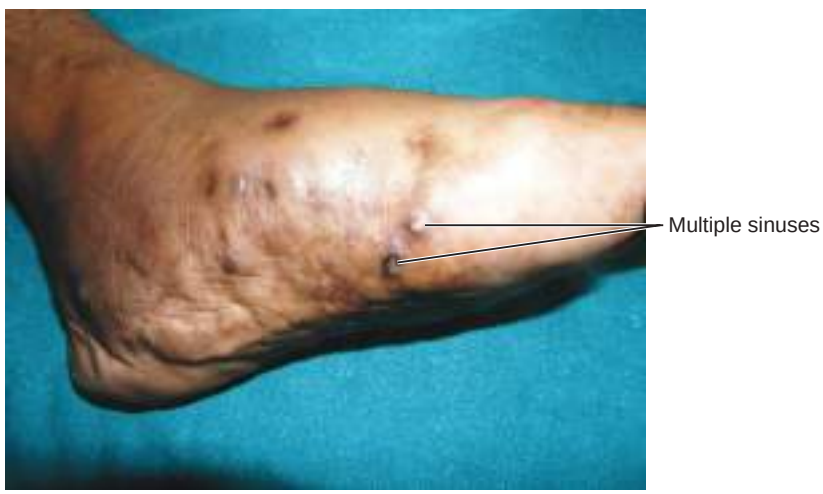


FIGURE 7.13. Mycetoma foot showing numerous draining sinuses.

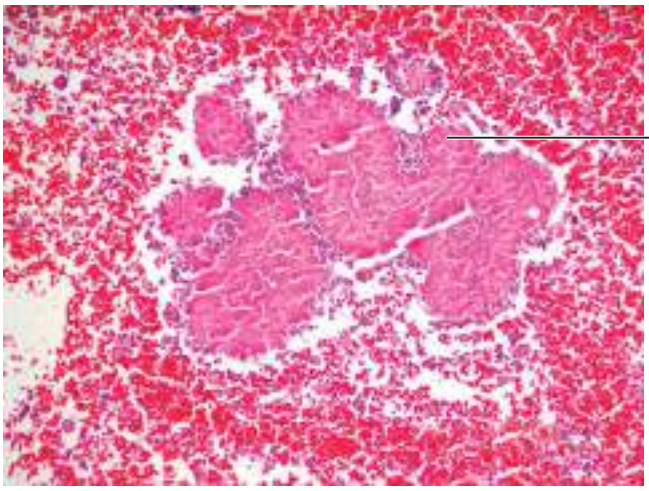


FIGURE 7.14. H&E-stained section showing a eumycetoma.

Laboratory Diagnosis

- **Direct microscopy:** Microscopic examination of crushed granules can be done using either 10% KOH and Parker ink, or calcofluor white mounts.
- **Tissue sections** can be stained using Gram's stain, H&E (Fig. 7.14), PAS and Grocott's methenamine silver (GMS).
- **Culture:** Clinical specimens should be inoculated onto primary isolation media, like Sabouraud's dextrose agar.

Q. Write briefly about protozoal infections.

Ans. Parasitic protozoa are single-celled eukaryotes that are major causes of disease and death in developing countries. They can replicate intracellularly within a variety of cells (eg, *Plasmodium* in red blood cells, *Leishmania* in macrophages) or extracellularly in urogenital system, intestine or blood.

Malaria

- Malaria is transmitted by female *Anopheles* mosquito. It is caused by parasites of the species *Plasmodium* that spread from person-to-person through bites of infected mosquitoes.
- The common first symptoms are fever, headache, chills and vomiting, and these appear 10–15 days after a person is infected. If not treated promptly with effective medicines, malaria can cause severe illness that is often fatal.
- There are four types of human malaria caused by *P. falciparum, P. vivax, P. malariae* and *P. ovale,* respectively. *P. falciparum* and *P. vivax* are the most common. *P. falciparum* is by far the most deadly type of malaria. The following are the features unique to *P. falciparum:*
  - High parasitemia
  - Severe anaemia
  - Frequent occurrence of renal failure, pulmonary oedema and death
  - *P. falciparum* causes RBCs to clump together (rosetting) and sticks to endothelial lining
  - Several proteins including *P. falciparum erythrocyte membrane protein* (*PfEMP1*) form knobs on surface of the RBCs. PfEMP1 binds to ligands on endothelial cells including CD36, thrombospondin, VCAM1, ICAM1 and E-selectin. This causes ischaemia, which is responsible for manifestations of cerebral malaria.
- Features common to P. vivax and P. malariae include
  - Mild anaemia
  - Splenic rupture
  - Nephrotic syndrome

Life Cycle (Flowchart 7.4)

Sporozoites reach blood by bite of an infected mosquito (female *Anopheles*)

↓

They invade and attach to liver cells (binding to hepatocytic receptors for proteins thrombospondin and properdin)

↓

Multiply asexually in the liver cells — ***Hepatic phase***

↓

Merozoites (asexual haploid forms) released into circulation when hepatocytes rupture

↓

Bind to RBCs via lectin on glycophorin molecules of RBC membrane (*P. vivax* binds to Duffy Ag)

↓

Merozoites release proteases from a special organelle called *Rhoptry* — ***Erythrocytic phase***

↓

Multiply in RBCs, within which parasites grow in membrane-bound digestive vacuoles and hydrolyse haemoglobin via serotonin enzymes, converting haem to haemozoin

Note: First stage of parasite in RBCs is* ring stage, followed by trophozoite *and schizont form (Figure 7.15).

Some merozoites develop into sexual forms called *gametocytes that infect mosquito when it takes a blood meal*

↓

Once ingested, the parasite gametocytes taken up in the blood will further differentiate into *male or female gametes* *(Fig. 7.16)* and then fuse in the mosquito gut

↓

This produces an *ookinete* that penetrates the gut lining and produces an *oocyst* in gut wall

↓

When the oocyst ruptures, it releases sporozoites that migrate through the mosquito's body to its salivary glands, where they are then ready to infect a new human host

FLOWCHART 7.4. Lifecycle of malarial parasite.

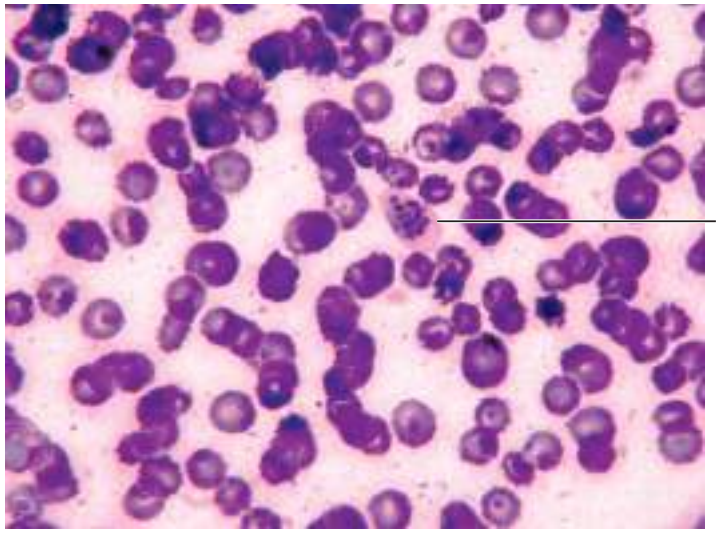


FIGURE 7.15. Schizont form of malarial parasite.

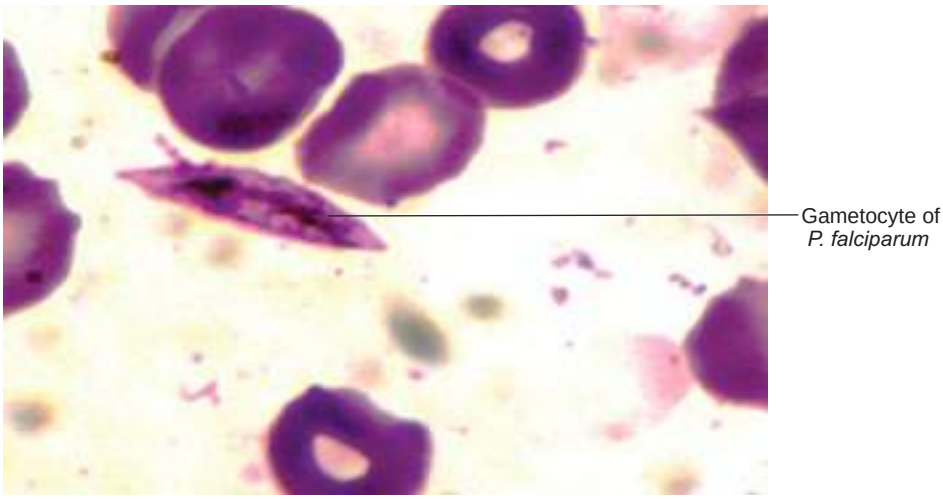


FIGURE 7.16. Peripheral smear showing gametocyte stage of *P. falciparum (arrows)*.

Resistance to Malaria Resistance to Malaria is Seen in Association With:

- Inherited alterations in RBCs (presence of HbS, HbC and absence of Duffy antigen)
- Repeated and prolonged exposure to *Plasmodium* species which stimulates an immune response that reduces the severity of malaria.

Histopathology

Spleen:

- Congested and enlarged (may exceed 1000 g in weight)
- Parenchyma is grey-black because of granular, brown-black, birefringent haemozoin pigment.
- Capsule is thickened and the spleen becomes extremely fibrotic and brittle *(fibrocongestive splenomegaly)*.
- Phagocytic activity of reticuloendothelial cells is increased.
- Liver is enlarged and sections show pigmented Kupffer cells, which are heavily laden with malarial pigment and parasite.
- Kidneys are enlarged with presence of pigment in glomeruli and haemoglobin casts in tubules.
- Nervous system:
  - Brain vessels plugged with parasitized RBCs, each with a dot of haemozoin.
  - Perivascular ring haemorrhages due to local hypoxia with vascular stasis and small focal inflammatory aggregates (**malarial or Durck granulomas**) is seen.
  - Also seen are degeneration of neurons and focal ischaemic softening.

Leishmaniasis

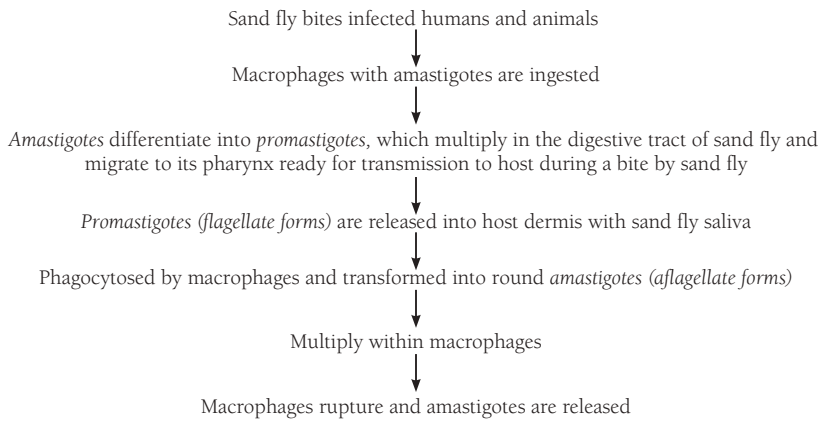
- Leishmaniasis is a chronic inflammatory disease of the skin, mucous membranes and viscera caused by an obligate intracellular protozoan transmitted through bites of infected sand fly.
- It is endemic throughout Middle East, South Asia, Africa and Latin America.
- Life cycle involves two forms: *promastigotes,* which develop and live extracellularly in the sand fly vector and the *amastigote* form that multiplies intracellularly in the host macrophages.
- Mammals including rodents, dogs and foxes are reservoirs of leishmania.
- Parasite specific cell-mediated immunity is the main host immune response.

The types of leishmaniasis are depicted in Table 7.6.

TABLE 7.6. Types of leishmaniasis

Type of disease	Causative species
Cutaneous disease	*L. major, L. mexicana, L. aethiopica, L. braziliensis*
Visceral pathology (kala azar)	*L. donovani, L. chagasi*

Life Cycle (Flowchart 7.5)



FLOWCHART 7.5. Life cycle of Leishmania.

- *Promastigotes* produce two surface glycoconjugates, important for their virulence, namely, *lipophosphoglycan* and *Gp63. Lipophosphoglycan* forms a dense glycocalyx, which activates complement to deposit C3b on the parasite surface, and inhibits complement by preventing membrane complex attack insertion into the parasite membrane.
- C3b coated on the parasite binds to Mac1 and CR1 on macrophages initiating promastigote phagocytosis by macrophages.
- *Lipophosphoglycan* neutralizes oxygen radicals and inhibits lysosomal enzymes, protecting the parasite in the phagolysosome.
- *Gp63*, a zinc-dependent proteinase that cleaves complement and some lysosomal antimicrobial enzymes; also promotes promastigote adhesion to macrophages.

Histopathology

- Invasion by parasite-laden macrophages throughout reticuloendothelial cells leads to systemic disease (hepatosplenomegaly, lymphadenopathy, pancytopenia, fever and weight loss).
- Phagocytic cells crowd the bone marrow, lymph nodes, liver, lungs, GIT, kidneys, pancreas and testes.
- Liver becomes fibrotic in later stages. Normal architecture of the spleen may be replaced by sheets of histiocytes, which are parasite laden. Plasma cells are increased in number.
- Kidney biopsy may show mesangioproliferative glomerulonephritis and/or amyloidosis.
- Hyperpigmentation of the skin *(black fever)* may be seen.

Cutaneous Leishmaniasis

- Usually manifests with a single ulcer on exposed skin *(tropical sore)*.
- Starts as a papule surrounded by induration, progresses to a shallow expanding ulcer with irregular borders, which usually heals by involution without treatment.
- Microscopy shows well-formed granulomatous reaction or ill-defined histiocytic aggregates with intracellular parasite.

Mucocutaneous Leishmaniasis

- Moist, ulcerating and nonulcerating lesions arise in larynx, nasal septum and vulva after the skin lesion has healed.
- Microscopy shows histiocytes, lymphocytes, plasma cells and occasionally granulomatous reaction.

Diffuse Cutaneous Leishmaniasis in Anergic Patients

Starts as a single nodule and spreads to the whole body as bizarre nodular lesions (resemble keloids and verrucae; they may sometimes be mistaken for the nodules of lepromatous leprosy).

Amoebiasis

- Amoebiasis is caused by *Entamoeba histolytica*, a protozoan parasite, which spreads by faecal-oral transmission. Amoeba proteins involved in tissue invasion include
  - *Cysteine proteases,* which lyse proteins of extracellular matrix.
  - *Lectins* on parasite surface that bind to carbohydrates on colonic epithelium and RBCs.
  - *Channel forming proteins* that contains an *ameba pore* that makes pores in plasma membrane and lyses it.
- It is important to distinguish the *E. histolytica* cyst from the cysts of nonpathogenic intestinal protozoa such as *Entamoeba coli.*
  - *E. histolytica* cysts have a maximum of four nuclei, while the commensal *E. coli* cyst has up to 8 nuclei.
  - In *E. histolytica,* the endosome is centrally located in the nucleus, while it is usually eccentric in *E. coli*.
  - Chromatoidal bodies in *E. histolytica* cysts are rounded, while they are jagged in *E. coli*.
  - Virulent strains of *E. histolytica* show ingested RBCs, whereas nonvirulent strains do not.

Life Cycle (Flowchart 7.6)

Ingestion of *cysts*
(Infectious forms of *E. histolytica* which have a chitinous wall and 4 nuclei and are resistant to gastric acids)
↓
Cysts colonize the mucin-secreting epithelial cells of colon
↓
In the colonic lumen, cysts release *trophozoites* or *amoeboid forms*
(lack of mitochondria and Krebs cycle enzymes makes them obligate fermenters of glucose to ethanol;
Metronidazole targets *pyruvate–oxido-reductase,* an enzyme critical in this fermentation)
↓
Dysentery, intestinal pain and fever

FLOWCHART 7.6. Life cycle of *E. histolytica*.

Pathology

- Caecum, ascending colon, sigmoid, rectum and appendix are involved in that order. In severe full blown cases, entire colon may be involved.
- Amoebae invade crypts of colonic glands and burrow through the wall up to muscularis mucosae (which acts as a barrier to the infection). Thereafter, they fan out laterally forming a *flask-shaped ulcer* (Fig. 7.17).
- The ulcer contains large areas of liquefactive necrosis and very few inflammatory cells. A sharp line divides the necrotic and viable mucosa. Trophozoites are found on the surface of the ulcers, in the exudate and in the crater. They are also frequently found in the submucosa, muscularis propria, serosa and small veins of the submucosa.
- **Amoeboma** is a napkin-like constrictive lesion (composed of granulation tissue), which may be confused with carcinoma colon.
- In 40% patients, parasites penetrate portal vessels leading to solitary and multiple abscesses in the liver **(amoebic liver abscesses)** filled with chocolate coloured, odourless,

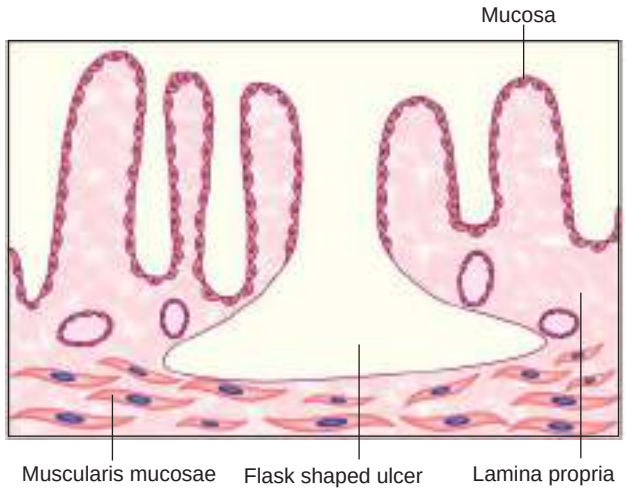


FIGURE 7.17. A flask-shaped amoebic ulcer.

pasty **(anchovy sauce-like)** material. These may undergo secondary bacterial infection causing suppuration.

Diagnosis

- Asymptomatic human infections are usually diagnosed by finding cysts shed in the stool. Various sedimentation procedures have been developed to recover the cysts from faecal matter.
- In symptomatic infections, the motile form (trophozoite) can often be seen in fresh faeces.
- Amoebic trophozoites can also be demonstrated in histopathology sections, where they appear as spherical or oval-shaped bodies (15–20 microns in diameter) with a thin cell membrane and single nucleus with prominent nuclear border and central karyosome. Trophozoites resemble macrophages because of a comparable size and presence of multiple vacuoles; the parasite, however, has a smaller nucleus with a large karyosome. The PAS procedure stains the cytoplasm of the trophozoite red. The organism appears black when stained with Heidenhain's iron haematoxylin method. Presence of trophozoites containing RBCs is indicative of tissue invasion.
- Serology becomes positive about 2 weeks after infection. The levels of antibody are much higher in individuals with liver abscesses.

Q. Write briefly about helminthic infections.

Ans. The helminths are worm-like, multicellular parasites. They undergo sexual reproduction in the definitive host and asexual multiplication in an intermediary host. The clinically important helminths are classified according to their physical characteristics, internal morphology (appearance of their egg, larval and adult stages), as well as, and the host/vector they inhabit. Flukes (Trematodes) are leaf-shaped flatworms with prominent oral and ventral suckers. Tapeworms (Cestodes) are elongated, segmented, hermaphroditic flatworms that inhabit the intestinal lumen. Larval forms, which are cystic or solid, inhabit extraintestinal tissues. Roundworms (Nematodes) are bisexual, cylindrical worms. They inhabit intestinal and extraintestinal sites.

Tapeworms (Cestodes): Cysticercosis and Hydatid Disease

- *Taenia solium* and *Echinococcus granulosus* are cestodes (tapeworms) that cause cysticercosis and hydatid infections, respectively. Both diseases are caused by larvae that develop following ingestion of tapeworm eggs.
- ***T. solium* tapeworms** consist of a head (scolex) that has suckers and hooklets that attach to the intestinal wall, a neck and many flat segments called proglottids that contain male and female reproductive organs.

- When pigs ingest the proglottids or eggs, the eggs hatch, penetrate their intestinal wall, and spread to skeletal muscle, especially the neck, tongue and trunk. There, the larvae mature into encysted cysticerci over 2–3 months.
- The cysticerci suppress the host inflammatory response and survive in tissues for months to years. The life cycle is completed when humans ingest inadequately cooked pork that contains viable cysticerci or eggs.
- The larvae hatch, penetrate the gut wall, disseminate haematogenously, and encyst in many organs. The egg-containing faeces usually contaminate water supplies in endemic areas. If this water is used to irrigate fruits and vegetables, eggs are ingested with the contaminated food. Thus, people who have never visited endemic countries can also develop infection.
- Autoinfection involves the retrograde transmission of proglottids from the intestines into the stomach with subsequent release of *T. solium* eggs into the gut.
- The **clinical syndromes caused by *T. solium*** are categorized as **neurocysticercosis** or **extraneural cysticercosis** (intestinal infection, subcutaneous nodules [Fig. 7.18] and ocular cysts). Neurocysticercosis can manifest with convulsions and other neurological signs of increased intracranial pressure.
- Neurocysticercosis is further divided into parenchymal and extraparenchymal disease. Parenchymal disease is characterized by infection within the brain parenchyma. Extraparenchymal disease develops when cysticerci migrate to the CSF of the ventricles, cisterns and subarachnoid space or within the eyes or spinal cord.

Hydatid Disease

- It is caused by ingestion of eggs of echinoccal species. Of the four known species of *Echinococcus*, three are of medical importance in humans. These are *Echinococcus granulosus* (causes cystic echinococcosis); *Echinococcus multilocularis* (causes alveolar echinococcosis) and *Echinococcus vogeli*. *E. granulosus* is the most common of the three. *E. multilocularis* is rare but is the most virulent, and *E. vogeli* is the rarest.
- Humans are accidental intermediate hosts for echinococcus, infected by ingestion of food contaminated with eggs shed by dogs or foxes.
- Eggs hatch in the duodenum and the larvae penetrate the intestine and disseminate haematogenously to encyst the liver, lungs or bones. Unilocular cysts caused by *E. granulosus* are most common. Multilocular cysts are caused by *E. multilocularis*. The cysts are ovoid and white to opalescent, rarely exceeding 1.5 cm, and contain an invaginated scolex with hooklets that are bathed in clear cyst fluid (Fig. 7.19).
- The cyst wall evokes little host reaction when it is intact. When cysts degenerate, however, there is inflammation, followed by scarring, and calcifications, which may be visible by radiography.

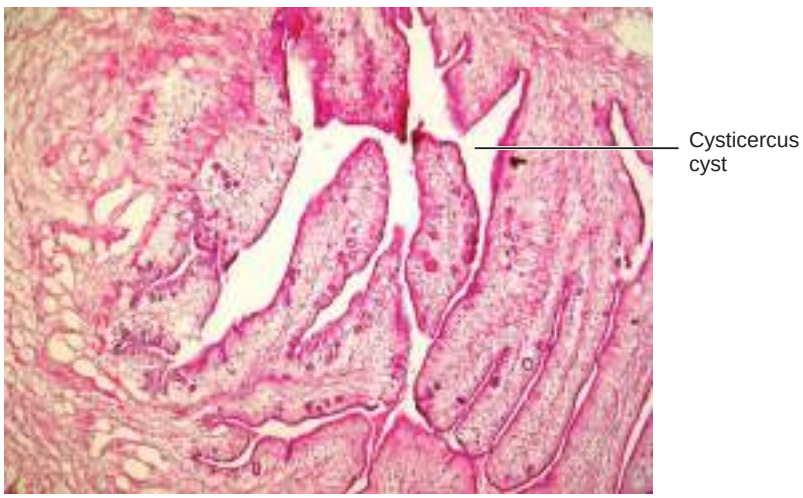


FIGURE 7.18. Cysticercosis (H&E; 100×).

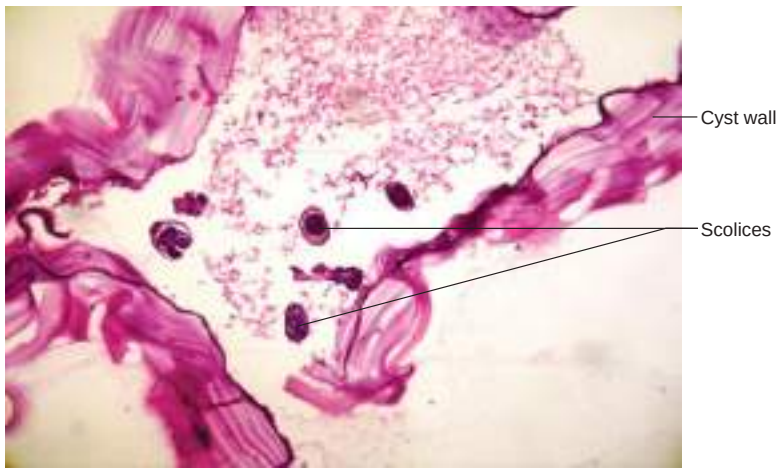


FIGURE 7.19. Echinococcosis liver showing invaginated scolices embedded in the cyst wall (H&E; 200×).

- About two-thirds of human *E. granulosus* cysts are found in the liver, 5–15% in the lung, and the rest in bones and brain or other organs.
- In the various organs, the larvae lodge within the capillaries and incite an inflammatory reaction composed principally of mononuclear leukocytes and eosinophils. Many such larvae undergo encystation.
- The cysts (Fig. 7.18) have an inner, nucleated, germinative layer and an outer, opaque, nonnucleated layer. The outer nonnucleated layer is distinctive and has innumerable delicate laminations as though made up of many layers of gelatin. Outside this opaque layer, there is a host inflammatory reaction that produces a zone of fibroblasts, giant cells and mononuclear and eosinophilic cells. In time, daughter cysts develop within them.

8

Genetic and Paediatric Disorders

PART I: GENETIC DISORDERS

Q. Write briefly on the structure of a gene.

Ans. A gene is a specific sequence of nucleotides. It codes for a protein through a genetic code or sequence called *codon (Fig. 8.1)*.

- The boundaries of a gene are known as *start* and *stop codons*. The *start codon* decides when to initiate the protein synthesis and the *stop (termination) codon* decides when to end it.
- Human genes contain *exons* which are regions that contain the coding information that are both transcribed and translated into proteins and *introns* which are stretches between exons that do not code for a protein (noncoding region).
- On either side of a gene, there are noncoding regions called *flanking regions* that are responsible for the regulation of gene expression. They are called *regulatory regions*. These include *promoters* (regions which bind to transcription factors strongly or weakly), enhancers (regions that enhance the effects of a weak promoter) and silencers (regions that inhibit transcription).
- In the first stage of transcription, an enzyme called RNA polymerase binds to a **TATA** base sequence in the 5′-flanking region (at the 'front end' of the gene) adjacent to where transcription is initiated. There are other sequences in the region that serve as sites to which proteins that assist in transcription bind. This entire flanking region prior to the coding region of the gene is called the *promotor*.
- On the far end of the gene, past the coding region of introns and exons, is the 3′-flanking region which largely remains untranslated.
- Once an mRNA is transcribed from the DNA coding region of a gene, it goes through several processing steps before it leaves the nucleus to be translated in the cytoplasm.

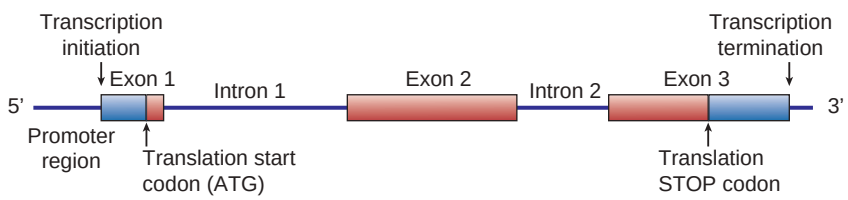


FIGURE 8.1. Structure of a gene.

Q. What are the different categories of genetic disorders?

Ans. Different categories of genetic disorders include the following:

1. Those related to single-gene mutations of large effect **(Mendelian disorders).**
2. Diseases with multifactorial **(polygenic)** inheritance, which involve both genetic and environmental influences **(complex multigenic disorders)**. They are caused by interaction between multiple variant forms of genes and environmental factors. These variations in genes are referred to as **'polymorphisms'**. Each variant gene causes a small increase or decrease in the risk of a disease. No single susceptibility gene is individually sufficient for inducing the disease. Several polymorphisms are required for the disease to occur.
3. Those arising from **structural** and **numeric aberrations** in autosomes and sex chromosomes **(chromosomal disorders).**

Q. Define mutation.

Ans. Mutation refers to a permanent change in DNA. Mutations which affect germ cells are transmitted to the progeny and may give rise to inherited diseases. Mutations in the somatic cells are not transmitted to the progeny and may give rise to cancers and congenital malformations.

- **Genome mutations** involve loss or gain of whole chromosomes giving rise to monosomy or trisomy.
- **Chromosome mutations** result from the rearrangement of genetic material to give rise to visible changes in the chromosome.
- **The most common mutations** associated with genetic disease are **gene mutations**, which involve partial or complete deletion of a gene or often a single base.

Examples:

- **Point mutations:** These can occur within coding sequences as well as noncoding sequences. The latter involve the regulatory sequences in the promoter/enhancer regions and not the exons. Point mutations result from the substitution of a single nucleotide base by a different base, resulting in the replacement of one amino acid by the other in the protein, eg, sickle cell anaemia. Such mutations alter the meaning of the genetic code and are thus called ***missense mutations.***
- Certain point mutations may change an amino acid codon to *a* ***chain termination codon*** or ***stop codon***. ***Nonsense mutations*** interrupt translation, leading to the formation of truncated proteins which are rapidly degraded.
- Point mutations or deletions involving regulatory sequences interfere with the binding of transcription factors leading to a gross reduction or complete absence of transcription (as seen in thalassaemias).
- Point mutations involving introns lead to defective splicing of intervening sequences.
- **Frame shift mutations** occur when the insertion or deletion of one or two base pairs alter the reading frame of the DNA strand.
- **Trinucleotide repeat mutations** are characterized by amplification of a sequence of three nucleotides; all affected sequences share guanine and cytosine, eg, in fragile X syndrome, there are 200–400 tandem repeats of the sequence CGG within a gene called FMR1 (familial mental retardation-1), which prevents the normal expression of FMR1 gene leading to mental retardation.

Q. Define pleiotropy.

Ans. The phenomenon in which a single gene mutation leads to many phenotypic effects is called pleiotropism, eg, **Marfan syndrome** is associated with widespread involvement of the connective tissue component of skeleton, eye, cardiovascular system, etc. all of which result from a single mutation in the **gene fibrillin**.

Q. Define genetic heterogeneity.

Ans. The phenomenon in which mutations at different genetic loci produce the same result is called genetic heterogeneity, eg, retinitis pigmentosa, a disorder of abnormal retinal pigmentation and visual impairment can be caused by several different types of mutations.

Q. Define aneuploidy.

Ans. Humans have 46 chromosomes consisting of 22 pairs of autosomes or somatic chromosomes and 2 sex chromosomes (XX = female and XY = male). The gametes contain a haploid number of chromosomes (n = 23). The union of two sex cells (egg and sperm), each with only haploid number of chromosomes, results in a diploid zygote (2n).

'**Hyperdiploidy' is a chromosomal number more than diploid and 'hypodiploidy' is a chromosomal number less than diploid. Aneuploidy** refers to the presence of an uneven multiple of 23 chromosomes. It is most frequently due to **nondisjunction**, in which one set of homologous chromosomes fails to separate during the first meiotic division (one gamete has 22 chromosomes and the other 24 chromosomes).

Q. What is chromosomal translocation?

Ans. Chromosomal translocation (Fig. 8.2) is the transfer of a broken segment from one chromosome to another nonhomologous chromosome. The process is usually reciprocal (fragments are exchanged between two chromosomes). Translocations are indicated by 't' followed by involved chromosome in numeric order.

- A special pattern of translocation involving two acrocentric (centromere at the end; q very long, p very short) chromosomes is called **centric fusion type** or **Robertsonian translocation.** The break occurs close to the centromere and affects the short arm of both chromosomes. Transfer of the segment leads to a very large and a very small chromosome. The short fragment is lost and the carrier has 45 chromosomes.
- **Isochromosomes** result when the centromeres divide horizontally and not vertically. One of the two arms of the chromosome is lost and the remaining is duplicated, resulting in a chromosome with two short arms or two long arms only.

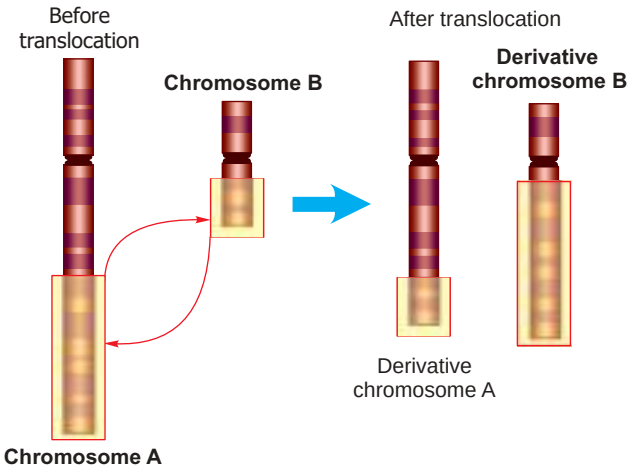


FIGURE 8.2. Chromosomal translocation.

Q. Define deletion.

Ans. Deletion is loss of a portion of a chromosome. A single break may delete a terminal segment. Two breaks with loss of an intervening segment is called an **interstitial deletion**. Two interstitial breaks with reunion of the proximal and distal segments may result in formation of a **ring chromosome.** After loss of segments from each end of the chromosome, the arms unite to form a ring.

Q. Define inversion.

Ans. Inversions occur when there are two interstitial breaks in a chromosome and the segment reunites after rotation.

Q. Define mosaicism.

Ans. When nondisjunction occurs during mitosis of autosomal cells, the result is mosaicism (presence of two or more genetically different cell populations in the same patient; a common occurrence in Turner syndrome).

Q. What are alleles?

Ans. Alternative forms of the same gene are called alleles. Genes with the same alleles are called homozygous, while those with different alleles are called heterozygous.

Q. Enumerate the cytogenetic disorders involving autosomes.

Ans. Cytogenetic disorders involving autosomes include the following:

1. Autosomal trisomies affecting chromosomes 21 (Down syndrome), 18 (Edwards syndrome) and 13 (Patau syndrome).
2. One deletion (cri du chat) syndrome due to partial deletion of short arm of chromosome 5 (characterized by mental retardation, a cat-like cry, and ventricular septal defects) .
3. Trisomies and deletions affecting 22q ('DiGeorge syndrome'—thymic hypoplasia, decreased T-cell immunity, parathyroid hypoplasia, hypocalcaemia; 'Velocardiofacial syndrome'—congenital heart disease, facial dysmorphism, delayed development).

Q. Write briefly on Down syndrome.

Ans. Down syndrome is a cytogenetic disorder affecting chromosome 21.

Incidence

1 in 700 live births.

Genetic Abnormalities

- Trisomy of chromosome 21 (47, XX, +21).
- Extra chromosome because of Robertsonian translocation of the long arm of chromosome 21 to another acrocentric chromosome like 22 or 14 [46, XX; der(14;21)(q10;q10); +21].
- Least common mosaic pattern having some cells with 46 chromosomes and some with 47 chromosomes due to mitotic nondisjunction of chromosome 21 during early stage of embryogenesis (46, XX/47, XX, +21).
- Trisomy of chromosome 21 is influenced by mother's age. Increased incidence is noted after 30 years of age.
- The extra chromosome is derived from nondisjunction of chromosome 21 during first meiotic division in ovum.

Clinical Features

- Mental retardation (IQ = 25–40).
- Flat facial profile and epicanthic folds, oblique palpebral fissure **(Mongolism/Mongolian idiocy).**
- Abundant neck skin.
- Congenital heart defects like atrial septal defects, endocardial cushion defects, atrioventricular valve malformations and ventricular septal defects (major cause of death during early life).
- Umbilical hernia.
- Intestinal stenosis.
- Hypotonia; gap between the first and second toe.
- Simian crease.
- Predisposition to acute leukaemia (lymphoblastic and myeloblastic).
- Neuropathological changes (like Alzheimer disease), seen after the age of 40 years.

Q. Write briefly on Edwards syndrome.

Ans. Edwards syndrome is a cytogenetic disorder affecting chromosome 18.

Incidence

1 in 8000 live births.

Genetic Abnormalities

Trisomy of chromosome 18 (47, XX, +18); mosaic type—46, XX/47, XX, +18.

Clinical Features

- Mental retardation
- Micrognathia
- Prominent occiput
- Low-set ears
- Short neck
- Congenital heart defects
- Renal malformations
- Limited hip abduction
- Overlapping of fingers
- Rocker bottom feet

Q. Write briefly on Patau syndrome.

Ans. Patau syndrome is a cytogenetic disorder affecting chromosome 13.

Incidence

1 in 15,000 live births.

Genetic Abnormalities

Trisomy of chromosome 13 (47, XX, +13); mosaic type—46, XX/47, XX, +13; translocation type—46, XX, +13, der(13;14)(q10;q10).

Clinical Features

- Microcephaly and mental retardation.
- Microphthalmia.
- Cleft lip and palate.

- Polydactyly.
- Congenital heart defects.
- Renal malformations.
- Umbilical hernia.
- Rocker bottom feet.

Q. Enumerate the cytogenetic disorders involving sex chromosomes.

Ans. Cytogenetic disorders involving sex chromosomes:

1. One X chromosome (maternal or paternal) may get randomly inactivated in the course of development (Lyon's hypothesis)
2. Klinefelter syndrome
3. Turner syndrome
4. Hermaphroditism and pseudohermaphroditism

Q. Write briefly on Lyon's hypothesis.

Ans. Lyon's hypothesis:

1. Only one of the X chromosomes is genetically active; the other X chromosome of either maternal or paternal origin undergoes hyperpyknosis and is rendered inactive.
2. Inactivation of either the maternal or paternal X chromosome occurs at random among all the cells of the blastocyst, on or about the 16th day of embryonic life.
3. Inactivation of the same X chromosome persists in all the cells derived from one precursor cell.
   (a) The inactivated X chromosome is selectively reactivated in germ cells before first meiotic division, as both X chromosomes are required for normal oogenesis ('Modified Lyon's hypothesis'—modification is based on the observation that women continue to express many genes from their inactive X chromosome).
   (b) The inactive X chromosomes can be seen in the interphase nucleus as the darkly staining small mass in contact with the nuclear membrane known as **Barr body** or X chromatin.
   (c) It is present in all cells of a normal female. A buccal smear is made to demonstrate it.
   (d) Normal females have one Barr body and normal males none.
   (e) A male with Klinefelter syndrome and an XXY genotype has one Barr body.

Q. Write briefly on Klinefelter syndrome.

Ans. Klinefelter syndrome is characterized by male hypogonadism due to presence of two or more X chromosomes and one or more Y chromosome.

Incidence

1 in 850 live male births.

Genetic Abnormalities

- Classical type 47, XXY karyotype (82% cases).
- Maternal nondisjunction is slightly more common than paternal nondisjunction of sex chromosomes; increases with increase in age.
- Other variants: 46, XY/47, XXY; 47, XXY/48, XXXY; may have more number of X chromosomes.

Clinical Features

- Distinctive body pattern and other features emerge after puberty.
- Elongated body due to increased length between the soles and the pubic bone.
- 'Eunuchoid body habitus': long legs, small atrophied testes and small penis.

- Loss of secondary male characters like deep voice, male distribution of pubic hair, beard and moustache and body hair.
- Gynaecomastia.
- No mental retardation but mean IQ lower than normal.
- One of the most common causes of *male infertility.*

Hormone Levels

- Increased follicle stimulating hormone (FSH)
- Low testosterone
- Increased estradiol

Microscopic Findings

- Features of testicular atrophy may be seen.

Q. Write briefly on Turner syndrome.

Ans. Complete or partial monosomy of X chromosome resulting in hypogonadism in the female phenotype.

Incidence

1 in 3000 female births.

Genetic Abnormalities

- Classic type: Entire X chromosome is missing (45, X).
- *Structural abnormality* of second X chromosome:
  - Deletion of small arm and formation of an isochromosome of the long arm—46, X, i(X) (q10).
  - Deletion of a portion of short and long arm, and formation of ring chromosome 46, X, r(X).
  - Deletion of a portion of short or long arm—46, X, del(Xq) or 46, X, del(Xp).
- *Mosaic pattern*: 45, X/46, XX; 45, X/46, XY; 45, X/47, XXX.

Pathogenesis

- During embryogenesis of ovaries, both X chromosomes are required.
- Normally fetal ovaries develop early in embryogenesis, but absence of second X chromosome leads to an accelerated loss of oocytes against a slow loss in normal female. By the age of 2 years, all the oocytes are destroyed.
- Ovaries are replaced by fibrous strands, with absence of ova and follicles (streak ovaries).

Clinical Features

- Infants present with peripheral oedema (lymph stasis) of the dorsae of hands and feet, and may have a swelling in the nape of neck (**cystic hygroma**) showing dilated lymphatic channels.
- With age, the swelling is replaced by bilateral neck webbing, loose skin on the back of neck and a low posterior hairline.
- Congenital heart diseases like coarctation of aorta and bicuspid aortic valve are the most common cause of death.
- Chest is broad and nipples are widely placed.
- Affected individuals may have streak ovaries (it is the single most important cause of primary amenorrhea), pigmented nevi, cubitus valgus and a short stature.
- Autoimmunity develops leading to hypothyroidism and glucose intolerance may also be seen.

Q. Describe disorders involving sex differentiation in both males and females.

Ans. An **XY karyotype** leads to differentiation of the primitive gonadal tissue into sex cords (seminiferous tubules) and Leydig cells, whereas an **XX karyotype** leads to preferential development of the germinal cortex into primordial follicles.

- A **true hermaphrodite** has both male and female gonads (ovary and testis).
- A **pseudohermaphrodite** is a person whose **phenotype** (appearance) is not in agreement with the **genotype** (true gonadal sex).
- A **male pseudohermaphrodite** is a genotypic male (XY with testes), who phenotypically resembles a female (eg, testicular feminization).
- A **female pseudohermaphrodite** is a genotypic female (XX with ovaries), who phenotypically resembles a male (eg, virilization in congenital adrenal hyperplasia).
- **Testicular feminization** is due to deficiency of androgen receptors (testosterone is unable to cause development of the seminal vesicles, epididymis) and vas deferens.

Q. Write briefly on Mendelian inheritance disorders.

Ans. Single gene defects (mutations) follow the Mendelian pattern of inheritance and are called Mendelian disorders. Mutations involving single genes usually follow one of the following three patterns of inheritance:

1. **Autosomal dominant (AD) disorders**
   (a) Only one abnormal allele is necessary to express the disease (manifests in the heterozygous state).
   (b) AD diseases are characterized by reduced penetrance, variable expressivity, and in some cases, late onset of the disease (eg, familial polyposis, Huntington chorea). Each affected individual has an affected parent unless the condition has arisen from a new mutation in the germ cells forming that individual.
   (c) Phenotypic expression of an inherited mutant gene or percentage carriers of the gene who express the trait is called **penetrance.** When some individuals inherit the mutant gene but are phenotypically normal (ie, a patient may have the abnormal gene but never expresses the disease), the trait is said to exhibit reduced penetrance.
   (d) If a trait is seen in all individuals carrying the mutant gene but they express the disease with different severity, it is called **variable expressivity** (eg, neurofibromatosis).
   (e) The manifestations of these disorders depend on the nature of protein affected and the type of mutation. 'Loss of function mutations' may affect proteins involved in control of complex metabolic pathways dependent on feedback regulation, eg, mutation in gene responsible for synthesis of low density lipoprotein (LDL) receptor results in decrease in the number of the same leading to increased cholesterol levels; or structural proteins like collagen, a reduction of which leads to skeletal abnormalities. 'Gain of function mutations' are less common and may lead to enhanced normal function of the protein, eg, increased activity of enzymes; or may induce a new function in addition to the normal function of the protein, eg, huntingtin in Huntington disease, which is neurotoxic to neurons.

   Examples: von Willebrand disease, familial hypercholesterolaemia, adult polycystic kidney, Huntington chorea, congenital spherocytosis, familial polyposis, neurofibromatosis and Marfan syndrome.

   Neurofibromatosis is associated with neurofibromas, iris hamartomas (Lisch nodules), café-au-lait spots, skeletal lesions (scoliosis) and an increased incidence of other tumours (acoustic neuromas, meningiomas, optic nerve gliomas and pheochromocytomas).

   Marfan syndrome, due to a defect in fibrillin, primarily affects the skeleton (eunuchoid habitus and arachnodactyly), eyes (dislocated lens) and cardiovascular system (mitral valve prolapse and dissecting aortic aneurysm).

2. **Autosomal recessive (AR) disorders**
   (a) Largest group of Mendelian disorders.
   (b) Both abnormal alleles must be present (homozygous state) to express the disease.
   (c) The trait does not always affect the parents but siblings may be affected.
   (d) The chance siblings getting affected is one in four.
   (e) May result from a consanguineous marriage.
   (f) The expression of the defect appears to be more uniform than in AD disorders.
   (g) Complete penetrance is common.
   (h) Onset is frequently early in life.
   (i) Because the affected individual may be an asymptomatic heterozygote, new mutations are rarely discovered clinically; several generations may pass before the descendants of such a person mates with other heterozygotes.

   Examples: Haemochromatosis, sickle cell anaemia, cystic fibrosis, Tay–Sachs disease, phenylketonuria, 21-hydroxylase deficiency, albinism, mucopolysaccharidoses (except Hunter syndrome), glycogenoses and galactosaemia.

   Lysosomal storage diseases are a group of diseases in which the absence of degrading enzymes leads to accumulation of complex substrates (eg, sphingolipids and mucopolysaccharides) in the lysosome.

   Glycogenoses involve accumulation of glycogen in tissue due to increased synthesis or decreased degradation of glycogen.
3. **X-linked disorders**
   (a) All sex-linked disorders are X-linked; no Y-linked diseases are known.
   (b) Most X-linked disorders are recessive.
   (c) They are transmitted by heterozygous female carriers only to their sons.
   (d) Heterozygous females rarely express the complete phenotype of the disease as they have the paired normal allele. Due to inactivation of one of the X chromosomes in females (Lyon's hypothesis), it is possible for the normal allele to be inactivated resulting in full expression of the disease in heterozygote females.
   (e) An affected male does not transmit the disease to his sons, but all his daughters are carriers.

   Examples: Lesch–Nyhan syndrome (hyperuricemia and self-mutilation due to deficiency of HGPRT), fragile X syndrome (mental retardation), haemophilia, glucose-6 phosphate dehydrogenase deficiency, testicular feminization, chronic granulomatous disease of childhood and Wiskott–Aldrich syndrome.

Q. Differentiate between the various Mendelian disorders.

Ans. Differences between the various Mendelian disorders are given in Table 8.1.

TABLE 8.1. Differences between the various Mendelian disorders

Features	Autosomal dominant	Autosomal recessive	Sex-linked recessive
Transmission	Both males and females are affected and can transmit the disease. Only one parent of the index case has the disease. 50% of children are affected and 50% normal	Both parents must be carriers (heterozygotes or homozygotes). 25% of children are symptomatic, 50% carriers and 25% normal	Males express the disease. Affected male transmits abnormal gene to 100% of his daughters (asymptomatic carriers), who then transmit the disease to 50% of their sons
Penetrance/variable expressivity/delayed onset of symptoms	Seen	Not seen	Not seen

Q. What is multifactorial inheritance?

Ans. Multifactorial (polygenic) inheritance disorders occur consequent to multiple small mutations plus the effect of environment.

Examples: Cleft lip or palate, congenital heart disease, coronary artery disease, gout, type II diabetes mellitus, hypertension, open neural tube defects and congenital pyloric stenosis.

Q. What are mitochondrial DNA disorders?

Ans. Mitochondrial DNA (mtDNA) disorders arise secondary to mutations in a mitochondrial genes, which primarily code for enzymes involved in oxidative phosphorylation.

- The disorders are unique to females (mitochondrial genes are inherited by maternal inheritance, since ova have more mitochondria than sperms which lose their mitochondria during fertilization).
- A female with an mtDNA defect transmits it to all her children.
- mtDNA in humans has 37 genes, of which 22 are transcribed into transfer RNAs and 2 into ribosomal RNAs. The remaining code for enzymes of oxidative phosphorylation pathway.
- Mitochondrial disorders, therefore, affect organs like CNS, skeletal and cardiac muscle, liver and kidney which are dependent on oxidative phosphorylation.
- Leber hereditary optic neuropathy, a neurodegenerative disease, is a prototype of mitochondrial disorders. It manifests with bilateral loss of central vision, cardiac conduction defects and neurological aberrations.

Q. Write briefly on the various molecular techniques used in pathology.

Ans. Hybridization is defined as the process of double-stranded molecule formation that occurs between target DNA or RNA and their complementary nucleic acid probes.

Probes

Segments of DNA or RNA labelled with radioisotope or nonradioisotope reporter molecules, which may be

1. Short single-stranded oligonucleotides.
2. Intermediate-sized complementary RNA probes.
3. Long double-stranded DNA probes.
   - Hybridization can be accomplished in *solution (polymerase chain reaction or PCR)*, on *solid support such as nitrocellulose or nylon membranes (Southern blot), or at the cellular or subcellular level (in situ hybridization)*.
   - Factors that affect the formation and stability of hybridization are composition of sequences and temperature and salt concentration. Higher temperature and low salt level lead to stringent hybridization, whereas low temperature and high salt concentration lead to relaxed hybridization with occasional mismatched base pair. Guanine cytosine (GC)-rich sequence forms a more stable product than an adenine cytosine (AC)-rich sequence because the former contains more hydrogen bonds that require a higher temperature to dissociate the hybrid structure. A direct relationship exists between DNA stability and its melting temperature.

Southern Blotting

In this filter hybridization method, denatured DNA is immobilized on an inert support that allows for the binding of a labelled nucleic acid probe.

Northern Hybridization (Blotting)

- This is a sensitive and quantitative method for mRNA detection.
- RNA is denatured with a variety of reagents. To obtain a high-quality RNA yield, it is important to avoid contamination and to inhibit RNAases during processing of tissue.
- Although RNA is single stranded, denaturation is required for effective separation on agarose gel.
- Denaturation is typically performed by using formamide with subsequent separation by electrophoresis on a formaldehyde gel.
- Separated RNA on the gel will be transferred to filter paper by capillary action and hybridized in a similar way, as Southern method, to a complementary target molecule.
- These are hybridized with radiolabelled probes.

Dot Blot Hybridization

This is a useful technique for quantitative measurement of target DNA sequences where the size of the target is known or is unnecessary. A membrane blotted with known DNA sequences will be hybridized with a test sample for the detection of a specific sequence.

Western Blotting

In this technique, electrophoretically separated components are transferred from a gel onto a solid support and probed with antibodies specific to certain epitopes on target protein.

In situ Hybridization

- The technique is ideal for visualization and localization of specific nucleic acid sequences in cells. A tissue sample or cell preparation mounted on a slide can be used as a target for probe hybridization.
- *In situ DNA*: The target for this technique is nuclear DNA and the probe sequences include centromeric, whole chromosome and specific gene.
- *In situ mRNA*: The target in this method is the mRNA transcript in the cytoplasm. The probes are manufactured in vitro by inserting a cloned DNA fragment of interest into a vector near a regulatory sequence (promoters) that direct RNA polymerase to transcribe sequence to RNA.
- A sense or antisense RNA probe can be generated depending on the orientation of inserted fragment. Each affected individual has an affected parent unless the condition has arisen from a new mutation in the germ cells forming that individual.
- The applications include gene localization and determination of translocation, ploidy and gene amplification.

Q. Write briefly on polymerase chain reaction (PCR).

Ans. PCR is an in vitro technique of nucleic acid synthesis that allows for rapid, sensitive and specific replication of nucleic acid for the detection and isolation of a targeted sequence.

- The method involves exponential amplification of DNA, and is based on the function of DNA polymerase enzyme to create a new complementary DNA strand from a template strand.
- If RNA is used as a substrate, it is first reverse transcribed to obtain cDNA and then amplified by PCR.
- The technique requires a pair of oligonucleotide primers that complement the opposite ends of each strand of a target sequence and are aligned for DNA synthesis to proceed only in the region between the primers.
- A reaction mixture for PCR amplifications typically contains a DNA sample, a pair of primers, thermostable Taq DNA polymerase enzyme and 4-deoxynucleotide triphosphates (dNTPs) in a buffered solution.

- For the reaction to proceed, target DNA is first denatured by heating and the reaction mixture then cooled to a certain temperature to allow the primers to anneal to the DNA target and, lastly, extension by Taq DNA polymerase is brought about.
- Repeating these cycles leads to exponential production of the specific target.

Steps

The repeated cycles of DNA polymerase activity include the following:

1. **Denaturation:** Heat is typically used to break the hydrogen bonds between complementary bases of both strands.
2. **Annealing:** This entails the binding of the primers to the beginning sequence of one strand and to the end of the other strand.
3. **Extension:** DNA polymerase and triphosphorylated deoxynucleotides are added to the reaction including the primers to extend the complementary strand.

Types

1. **Simple PCR:** This entails the amplification of a single specific sequence by using a pair of primers complementary to the flanking sequences of the target and is routinely used for preparing more DNA targets for subsequent analyses.
2. **Multiplex PCR:** In this technique, a number of different primers are added to the same reaction mixture to screen for multiple abnormalities and to avoid unnecessary testing.

Q. Write briefly on fluorescence in situ hybridization (FISH).

Ans. This is a method of identifying a chromosome or its parts by the use of specific probes that bind to specific DNA sequences (which are complementary).

- It is more useful than traditional karyotyping, as cells can be visualized even in interphase.
- It circumvents the need for dividing cells; even those cells that are not dividing or cannot be induced to divide can be mapped.
- The probes are attached with fluorescent dyes and are visualized under a fluorescent microscope.

Applications

- Karyotyping of cells/chromosomes in interphase.
- Using specific complementary DNA sequence, one can look for specific regions on a chromosome.
- Can be used for detection of numerical abnormalities, eg, aneuploidy, microdeletions and complex translocations that are not readily visualized by karyotyping.
- Mapping/localization of newly isolated genes of clinical importance.

Chromosome painting: It is visualization of the entire chromosome using different fluorescent dyes.
Spectral karyotyping: Using computer-generated signals the entire human genome can be 'painted' and visualized simultaneously.

Q. Write briefly on array comparative genomic hybridization.

Ans. Array comparative genomic hybridization, also called **CMA (chromosomal microarray analysis)** or **CGH (microarray-based comparative genomic hybridization),** is a technique to detect genomic variations, at a higher resolution level than chromosome-based CGH without prior knowledge of what these aberrations may be. DNA from a test sample and normal reference sample are labelled differentially, using different fluorescent dyes and hybridized to several thousand probes. The probes are derived from most of the known genes and noncoding regions of the genome, printed on a glass slide. The ratio of the fluorescence intensity of the test to that of the reference DNA is then calculated, to measure the copy number changes for a particular location in the genome.

Q. Write briefly on gene polymorphism.

Ans. Gene polymorphism is an occurrence in a population of two or more genotypes in frequencies that cannot be accounted for by recurrent mutation. Genetic variations occurring in more than 1% of a population would be considered useful **polymorphisms** for genetic linkage analysis. Such occurrences are generally long term. Genetic polymorphism may be balanced (such that allele frequencies are in equilibrium with one another at a given locus) or transient (such that a mutation is spreading through the population in a constant direction).

Q. What are SNP genotyping arrays?

Ans. Single nucleotide polymorphism (SNP) genotyping arrays are newer genomic arrays based on identification of SNPs sites genome wide. SNPs are the most common DNA polymorphisms which occur after every thousand nucleotides throughout the genome. SNP arrays are used to find copy number abnormalities when the karyotype is normal but a structural abnormality is suspected.

Q. Write briefly on the biochemical consequences of single gene Mendelian disorders.

Ans. The following mechanisms are involved in single-gene disorders:

1. Enzyme defects.
2. Defects in membrane receptors.
3. Abnormalities in the quantity, structure and function of nonenzymatic proteins.
4. Mutations leading to aberrant drug reactions.

Enzyme Defects

Mutations may result in the synthesis of a defective enzyme or synthesis of reduced quantity of a normal enzyme. The biochemical consequences of an enzyme defect in such a reaction may lead to three major consequences:

1. Defect in an enzyme in a major pathway may result in accumulation of the substrate or one or more intermediates. An increased level of one of the intermediates stimulates the minor pathway leading to an excess of other metabolites. Excess quantity of the substrate or the intermediates may lead to tissue injury, particularly if they are toxic in nature. For example, in galactosaemia, the deficiency of galactose-l-phosphate uridyltransferase leads to the accumulation of galactose and consequent tissue damage.
2. An enzyme defect can block a major pathway and lead to decreased amount of end product that may be necessary for normal function. For example, a deficiency of melanin may result from lack of tyrosinase, which is necessary for biosynthesis of melanin from its precursor, tyrosine leading to a clinical condition called albinism.
3. Failure to inactivate a tissue-damaging substrate, eg, α1-antitrypsin (α1-AT) deficiency. Patients who have an inherited deficiency of serum α1-AT are unable to inactivate neutrophil elastase in their lungs. This leads to destruction of elastin in the alveolar walls, resulting eventually in pulmonary emphysema.

Defects in Membrane Receptors

Defective receptor mediated transport as seen in familial hypercholesterolemia (decreased number or defective function of LDL receptors leads to impaired transport of LDL into cells and excessive cholesterol synthesis).

Abnormalities in the Quantity, Structure and Function of Nonenzymatic Proteins

Examples of genetic disorders with abnormalities in the quantity, structure and function of nonenzymatic proteins include haemoglobinopathies like sickle cell anemia. Other

proteins affected are collagen (osteogenesis imperfecta), dystrophin (muscular dystrophy), spectrin (spherocytosis), etc.

Mutations Leading to Aberrant Drug Reactions

Prototypical example is drug induced injury seen in glucose-6-phosphate deficiency. No haemolysis is seen in these patients under normal circumstances, however, administration of certain drugs like primaquine can result in severe haemolysis.

Q. Write briefly on enzyme defects and their consequences.

Ans. *Disorders associated with defects in enzymes include the following:*

1. **Phenylketonuria (PKU)**
   - PKU is characterized by deficiency of **phenylalanine hydroxylase**, which converts **phenylalanine** to **tyrosine.**
   - Infants are normal at birth but they develop increased phenylalanine levels within a few weeks.
   - Rising phenylalanine levels impair development of the brain, leading to severe mental retardation.
   - Other clinical features are seizures, decreased pigmentation of the hair and skin, eczema and strong mousy or musty odour of sweat and urine (due to accumulation of minor pathway products).
2. **Galactosaemia**
   - Galactose comes from the metabolism of lactose (glucose + galactose). In galactosaemia, there is a total lack of **galactose-1-phosphate uridylyltransferase (GALT)** leading to accumulation of glucose-1-phosphate and galactose.
   - Galactose-1-phosphate is toxic and damages tissue resulting in neonatal cholestasis (may progress to cirrhosis), CNS damage (mental retardation), renal damage (aminoaciduria) and *Escherichia coli* sepsis. Excess galactose may be converted into polyol (alcohol sugar), which causes osmotic damage to the lens, nerve tissue, liver and CNS.
3. **Homocystinuria**
   Homocystinuria is due to deficiency of **cystathionine synthetase.** It resembles Marfan syndrome (shares arachnodactyly and a dislocated lens). Differentiating features from Marfan syndrome include mental retardation, thromboembolic episodes (homocysteine damages endothelial cells) and osteoporosis.
4. **Alkaptonuria**
   - Alkaptonuria (ochronosis) is secondary to a lack of **homogentisic oxidase** required for the metabolism of phenylalanine.
   - There is an increase in homogentisic acid in urine, which is colourless at first but turns black after oxidation, upon exposure to light.
   - Homogentisic acid binds to collagen in connective tissue, tendons and cartilage (causing a crippling joint disease), and imparts a black colour to all these tissues.
5. **Lysosomal storage diseases** (See question on "Lysosomal storage diseases").
6. **Glycogen storage diseases** (See question on "Glycogen storage diseases").

Q. Write briefly on lysosomal storage diseases.

Ans. Lysosomes contain a variety of hydrolytic enzymes that are involved in degradation of complex substrates, eg, sphingolipids and mucopolysaccharides, into soluble end products. Due to a lack of a lysosomal enzyme, catabolism of a substrate remains incomplete leading to accumulation of the partially degraded insoluble metabolites within the lysosomes.

- Approximately 40 lysosomal storage diseases have been identified.
- Lysosomal storage diseases have an AR transmission and commonly affect infants and children.

- Accumulation of insoluble intermediates may lead to the following:
  - Organ enlargement (hepatosplenomegaly or cardiomegaly).
  - CNS involvement (neuronal damage).
  - Macrophage activation and cytokine release aiding to widespread cellular dysfunction
- Traditionally, lysosomal storage diseases are classified based on the biochemical nature of the substrate or accumulated metabolite.

Examples:

- **Tay–Sachs disease: G_{M2}** gangliosidosis
  - Enzyme deficiency: lysosomal hexosaminidase (α-subunit).
  - Metabolite accumulation: G_{M2} ganglioside.
  - It is primarily seen in Ashkenazi Jews.
  - Patients are normal at birth but develop signs of severe mental retardation within 6 months.
  - There is blindness, a cherry-red spot in the macula, muscle weakness, flaccidity and death by 2–3 years.
  - Histopathology shows ballooned neurons with cytoplasmic vacuoles which are actually distended lysosomes containing gangliosides which stain with fat stains like oil red O and Sudan black B. Retinal ganglion cells show the same changes. The normal colour of the macular choroid appears exaggerated due to the pallor of the adjacent swollen ganglion cells resulting in the 'cherry-red spot'.
- **Niemann–Pick disease**
  - Enzyme deficiency: sphingomyelinase.
  - Metabolite accumulation: sphingomyelin, primarily in macrophages (imparting a bubbly appearance) and in neurons.
  - Three variants: A, B and C; in the more severe type A, there is severe neuronal damage and mental retardation, massive hepatosplenomegaly and deterioration of psychomotor function (disease is fatal in early life).
  - In type B, neuronal damage is not present.
  - Type C was initially thought to be a variant of types A and B but is now considered a distinct clinicopathological and genetic entity. It is caused by mutations in two closely related genes—NPC1 and NPC2. Niemann–Pick type C is due to a primary defect in free cholesterol transport from the lysosomes to the cytoplasm and resultant accumulation in different organs especially in the nervous system. Patients have ataxia, dysarthria and psychomotor regression.
- **Gaucher disease**
  - AR disorder; most common lysosomal storage disease.
  - Enzyme deficiency: glucocerebrosidase or glucosylceramidase (primarily noted in Ashkenazi Jews).
  - Normally, the glycolipids derived from the breakdown of senescent blood cells are sequentially degraded (glucocerebrosidase cleaves glucose from ceramide). In Gaucher disease, the degradation stops at the level of glucocerebroside, which accumulates in the macrophages and CNS. Adverse results of Gaucher disease are caused not only by the accumulated glucocerebroside but also due to activation of macrophages which release various cytokines (IL 1, IL 2 and TNF).
  - The most common, type I (chronic nonneuronopathic form), accounts for 99% of cases of Gaucher disease. The glucocerebrosides accumulate in the mononuclear phagocytic system without affecting the CNS. Distended lysosomes give the macrophages a characteristic **fibrillary** or **wrinkled tissue paper appearance (**called **'Gaucher cells')**. There is massive hepatosplenomegaly, skeletal involvement (producing bone erosions and fractures), involvement of bone marrow (producing pancytopenia) and lymphadenopathy.
  - Types II (acute neuronopathic type) and III (intermediate between types I and II) have variable CNS involvement. Type II disease shows no preferential involvement of Jews and has an infantile acute cerebral pattern of presentation. Progressive CNS involvement leads to an early death. Type III disease shows systemic involvement with CNS disease which begins late in adolescence or adulthood.
- **Metachromatic leukodystrophy**
  - Enzyme deficiency: arylsulfatase A.

- Metabolite accumulation: sulfatide.
- The myelin that is synthesized is abnormal, hence affecting the CNS and peripheral nerves.
- There is mental retardation, peripheral neuropathy and visceral organ abnormalities.

- **Krabbe disease**
  - Enzyme deficiency: galactosylceramidase.
  - Metabolite accumulation: galactocerebrosidase.
  - Similar to metachromatic leukodystrophy, there is synthesis of an abnormal myelin leading to progressive psychomotor retardation.
  - Sections from brain at autopsy reveal multinucleated globoid cells loaded with the galactocerebroside material.
- **Fabry disease**
  - Enzyme deficiency: α-galactocerebrosidase A
  - Metabolite accumulation: ceramide trihexoside
  - It is characterized by angiokeratomas on the skin, hypertension and renal failure (X-linked recessive disease)
- **Mucopolysaccharidoses**
  - Mucopolysaccharides form a part of the ground substance synthesized by connective tissue fibroblasts, a certain fraction of which is degraded within lysosomes.
  - Mucopolysaccharidoses is characterized by accumulation of mucopolysaccharides due to lack of certain enzymes involved in their catabolic pathway.
  - Several clinical variants (MPS I to MPS VII) are known.
  - **Two well-recognized syndromes belonging to this category:**
    - Hurler syndrome (part of MPS I)
      - Enzyme deficiency: α-L-iduronidase.
      - Metabolite accumulation: dermatan sulphate and heparin sulphate.
      - Patients have severe mental retardation, coarse facial features, massive hepatosplenomegaly, clouding of the cornea, a high incidence of coronary disease owing to accumulation of the metabolites in the coronary vessels, joint stiffness and vacuoles in leukocytes in the peripheral blood.
    - Hunter syndrome (part of MPS II)
      - X-linked inheritance
      - Enzyme deficiency: L-iduronate sulfatase
      - Metabolite accumulation: dermatan sulphate and heparin sulphate
      - Absence of corneal clouding and a milder course differentiates it from Hurler syndrome

Q. Write briefly on glycogen storage diseases or glycogenoses.

Ans. Principal groups of glycogenoses are given in Table 8.2.

Q. Enumerate the different teratogens and write briefly on their effects.

Ans. Teratogens may be classified as follows:

1. **Noninfectious teratogens**
   (a) ***Alcohol*:** Fetal alcohol syndrome occurs in the offspring of women who have more than 4–6 drinks per day. It results in intrauterine growth retardation, maxillary hypoplasia, mental retardation, microcephaly, atrial septal defects and hypoglycaemia at birth.
   (b) ***Smoking*:** Associated with low birthweight and sudden infant death syndrome, smoking can lead to spontaneous abortions and placental abnormalities.
   (c) ***Cocaine*:** Cocaine can cause abruptio placentae and premature labour in the mother and CNS infarcts, intraventricular haemorrhage, genitourinary and gastrointestinal abnormalities in the newborn.
   (d) ***Isotretinoin*:** Used to treat acne; it may induce craniofacial abnormalities (small ears, micrognathia and cleft palate), cardiac defects and CNS malformations (microcephaly).

TABLE 8.2. Principal groups of glycogenoses

Clinicopathological category	Specific type	Enzyme deficiency	Morphological changes	Clinical features
Hepatic type	Hepatorenal (von Gierke disease)	Glucose-6-phosphatase	Hepato and renomegaly: intracytoplasmic accumulation of glycogen	Failure to thrive, stunted growth, hypoglycaemia, hyperuricaemia, hyperlipidaemia
Myopathic type	McArdle disease (type V)	Muscle phosphorylase	Skeletal muscle: accumulation of glycogen in the sarcolemmal location	• Painful cramps, myoglobinuria • No increase in lactic acid with exercise
Miscellaneous	• Generalized glycogenosis • Pompe disease (type II)	Lysosomal glucosidase (acid maltase)	Mild hepatomegaly, cardiomegaly, deposits in skeletal muscle	• Massive cardiomegaly, muscle hypotonia, cardiorespiratory failure within 2 years • Milder adult form with only skeletal muscle involvement, presents with chronic myopathy

(e) ***Diethylstilbestrol (DES)*:** DES causes abnormalities in Mullerian structures, eg, vaginal adenosis and is a precursor of clear cell adenocarcinoma of cervix.
(f) ***Thalidomide*:** It is associated with limb abnormalities: amelia (absent limbs) and phocomelia (seal-like limbs).
(g) ***Phenytoin*:** Consumed during pregnancy, phenytoin is associated with hypoplasia of the distal phalanges, CNS abnormalities and cleft lip/palate.
(h) ***Diabetes mellitus*:** Children of diabetic mothers may manifest with increased birthweight (macrosomia), open neural tube defects, cleft lip/palate, respiratory distress syndrome and transposition of great vessels.

2. **Infectious teratogens**
   (a) ***Cytomegalovirus (CMV)***
      (i) *Most common in utero viral infection.*
      (ii) Primarily transplacental transmission.
      (iii) May manifest with hearing loss, periventricular calcification, neonatal cholestasis, anaemia, thrombocytopenia, chorioretinitis (blindness) and microcephaly.
      (iv) Virus can be isolated from urine, saliva, blood and tissue. Histopathology reveals basophilic intranuclear inclusions labelled 'owl's eye appearance'.
   (b) ***Rubella***
      (i) Primarily transplacental transmission.
      (ii) Manifests with nerve deafness (most common defect), congenital heart disease (patent ductus arteriosus), cataract and mental retardation.
      (iii) Positive serologic test (TORCH) indicates disease.
   (c) ***Toxoplasmosis***
      (i) Maternal infection secondary to exposure to cats.
      (ii) Primarily transplacental transmission.
      (iii) Manifests with chorioretinitis (blindness), periventricular calcifications, microcephaly, mental retardation and neonatal cholestasis.
      (iv) Positive serologic test (TORCH) indicates disease.
   (d) ***Herpes simplex***
      (i) Primarily perinatal transmission while passing through birth canal with active shedding of herpes genitalis (HSV-2 baby should be delivered by caesarean section if viral shedding present).

(ii) Permanent neurological sequelae common.
(iii) Positive serologic test (TORCH) indicates disease.

(e) ***Syphilis***
(i) Primarily transplacental transmission.
(ii) Symptoms and signs appear in first 1–2 months after birth and include mucocutaneous lesions, lobar pneumonia, persistent rhinitis (snuffles), osteochondritis and hepatomegaly.
(iii) Prominent late manifestations are bone abnormalities (saber shins) and Hutchinson's triad (malformed, notched upper central incisors, interstitial keratitis leading to blindness and nerve deafness).
(iv) Rising VDRL titer; positive FTA-ABS-IgM are diagnostic.

Q. Write briefly on the disorders associated with prematurity.

Ans. *Newborns may be classified as appropriate for gestational age (AGA), small for gestational age (SGA) or large for gestational age (LGA).*

- *Term newborns are those born between 37 and 42 weeks, pre-term newborns are those born before 37 weeks and post-term newborns are those born after 42 weeks.*
- Infants born before completion of gestation usually weigh less than normal (2500 g).
- Prematurity is second only to congenital malformations as a cause of infant mortality.
- Preterm infants have immature organs, which predisposes them to various complications, eg, immature lungs (that lack surfactant and are prone to develop respiratory distress syndrome), necrotizing enterocolitis and intraventricular haemorrhage.

PART II: DISEASES OF INFANCY AND CHILDHOOD

Q. Write briefly on neonatal respiratory distress syndrome/hyaline membrane disease.

Ans. Characterized by formation of pulmonary hyaline membrane, neonatal respiratory distress syndrome is associated with the following conditions:

- Preterm infants.
- Infants born to diabetic mothers.
- Caesarean section delivery.
- Excessive sedation of mother during labour.
- Other birth-related asphyxias.

Aetiopathogenesis *(Flowchart 8.1):*

Decreased alveolar surfactant
↓
Increased alveolar surface tension and atelectasis
↓
Uneven perfusion and hypoventilation leading to hypoxaemia and CO_2 retention
↓
Acidosis and further reduction in surfactant synthesis
↓
Pulmonary vasoconstriction and hypoperfusion
↓
Endothelial and epithelial damage
↓
Leakage of plasma into alveoli
↓
Accumulation of fibrin and necrotic cells
↓
Hyaline membrane formation

FLOWCHART 8.1. Aetiopathogenesis of hyaline membrane disease.

Clinical Features

- Dyspnoea, tachypnea, hypoxia and cyanosis.
- May be fatal in severe cases.

Morphology

***Gross*:** Lung or its part(s) are normal in size, solid, airless, reddish purple in colour and sink(s) in water.
***Microscopic features*:** Collapsed alveoli, neutrophilic infiltration, eosinophilic hyaline membrane in the terminal bronchioles and alveolar ducts/alveoli.

Q. Define fetal hydrops. Enumerate and describe the two types of hydrops.

Ans. Fetal hydrops refer to accumulation of oedema fluid in the fetus during intrauterine growth

Fluid accumulation may vary from progressive generalized oedema of the fetus (hydrops) to a more localized isolated pleural/peritoneal collection
It may be *immune* or *nonimmune* in origin.

1. **Immune hydrops (erythroblastosis fetalis; haemolytic disease of newborn):**
   - Haemolytic disease in the newborn is caused by blood group incompatibility between mother and child.
   - Occurs when the fetus inherits red cell antigenic determinants from the father that are foreign to the mother.
   - Immunization of the mother by blood group antigens on fetal red cells and the free passage of antibodies from the mother through the placenta to the fetus is the basis of the disease.
   - Antigens may reach maternal circulation in the last trimester when the cytotrophoblast is no longer present as a barrier or during childbirth (fetomaternal bleed).
   - Most common incompatibility is Rh (D), followed by ABO blood group:

     Rh incompatibility:
     - When the mother is Rh-negative and the fetus is Rh-positive, the first child is usually unaffected; but all pregnancies in the future producing an Rh-positive fetus will be affected. Immunoglobulin containing anti-D antibodies should be administered within 72 h of delivery and/or at 28th week of pregnancy to Rh-negative mothers to prevent complications in the subsequent pregnancies.
     - If an Rh-negative mother has already been sensitized by Rh-positive blood due to prior transfusion, even the *first* Rh-positive child may be affected.
     - Such sensitized mothers form antibodies against Rh antigens.
     - Antibodies (Abs) cross placenta during the first pregnancy but usually in late third trimester; IgM isotype is the first antibody to be formed, which does not cross placenta, so the first child is mostly unaffected; but if there is heavy and early sensitization of mother, then haemolytic symptoms are visible in the first child due to IgG formation (which is capable of crossing placenta).
     - Second Rh-positive fetus causes large amount of IgG antibody formation. These antibodies cross placenta and attach to Rh-positive fetal RBCs.
     - Destruction of such RBCs leads to anaemia and haemolytic jaundice. In severe cases, jaundice may lead to kernicterus and mental retardation; anaemia may cause extramedullary hematopoiesis and/or cardiac decompensation leading to *hydrops fetalis*.

     ABO incompatibility:
     - This is less common compared to *Rh incompatibility* because anti-A and anti-B antibodies are IgM type, they do not cross the placenta.
     - Neonatal RBCs express blood group antigens A and B poorly, resulting in less sensitization of the mother.
     - ABO haemolytic disease occurs exclusively in infants born to 'O' blood group mothers (IgG type anti-A and anti-B antibodies).

2. **Nonimmune hydrops**
   - Major causes include cardiovascular defects, chromosomal anomalies (Turner syndrome, trisomies 18 and 21) and fetal anaemia (eg, that associated with homozygous α-thalassaemia) resulting in intrauterine cardiac failure.
   - Transplacental infection with parvovirus B19 is emerging as an important cause of fetal hydrops.

Morphology of Hydrops

- Presence of dysmorphic features suggests underlying chromosomal abnormalities.
- Postmortem examination may reveal a cardiac anomaly.
- In hydrops associated with fetal anaemia, both the fetus and the placenta are characteristically pale.
- In most cases, there is hepatosplenomegaly.
- Compensatory erythroid hyperplasia may be seen in the marrow and extramedullary haematopoiesis may be seen in liver, spleen, kidneys and lungs.
- Haemolysis leads to increased unconjugated bilirubin.
- CNS is damaged when bilirubin levels are more than 20 mg/dL. Basal ganglia and brain stem are prone to deposition of bilirubin *(Kernicterus)*.

Q. Enumerate the common malignant tumours of infancy and childhood.

Ans. Common malignant tumours of infancy and childhood are enlisted in Table 8.3.

TABLE 8.3. Common malignant tumours of infancy and childhood

0–4 years	5–9 years	10–14 years
Leukaemia	Leukaemia	Hepatocellular carcinoma
Retinoblastoma	Retinoblastoma	Soft-tissue sarcomas
Neuroblastoma	Neuroblastoma	Osteogenic sarcoma
Wilms tumour	Hepatocellular carcinoma	Thyroid carcinoma
Hepatoblastoma	Soft-tissue sarcomas	Hodgkin disease
Soft-tissue sarcomas	CNS tumours	
Teratomas	Ewing sarcoma	
CNS tumours	Lymphoma	

Q. Enumerate the small round blue cell tumours of childhood.

Ans. Many childhood tumours are collectively termed 'small round blue cell tumours of childhood' because they have a similar histological appearance, that is, presence of small round cells with a high N/C ratio. Subtle morphological clues may be present to distinguish between the tumours assisted by immunohistochemistry, electron microscopy and molecular analysis for chromosomal abnormalities. Following is a list of the most common tumours placed in this category:

- Ewing sarcoma and primitive neuroectodermal tumour
- Small cell osteosarcoma
- Leukaemia–lymphoma
- Neuroblastoma
- Rhabdomyosarcoma
- Wilms tumour
- Retinoblastoma
- Medulloblastoma
- Desmoplastic small round blue cell tumour

Q. Describe the clinicopathological features of neuroblastoma.

Ans. Enlisted below are the important clinicopathological features of neuroblastoma:

Clinical Presentation

- Second most common solid malignancy of childhood after brain tumours; most common in infants (<1 year of age).
- Mostly sporadic, rarely familial with AD transmission.
- *Most common site* is adrenal medulla; *other sites*—anywhere along sympathetic chain (paravertebral region of the posterior mediastinum and lower abdomen).
- Familial cases are associated with germline mutations in the anaplastic lymphoma kinase (ALK) gene. Somatic gain of function mutations also seen in less than 10% cases (inhibitors of this kinase are being used as potential treatment for neuroblastoma).

Gross Morphology

- Neuroblastomas vary from being in situ lesions (small nodule) to large masses.
- In situ lesions may regress to leave only small foci of fibrosis and calcification (spontaneous regression or therapy-induced maturation).
- Some tumours appear encapsulated and sharply demarcated, while others are highly infiltrative.
- Cut surface is soft, grey, brain-like with areas of necrosis and haemorrhage.

Microscopic Features

- Tumour is constituted by small, primitive-looking cells having dark nuclei, scant cytoplasm and poorly defined cell margins arranged in sheets.
- Mitotic activity, nuclear breakdown (karyorrhexis) and pleomorphism is prominent.
- Eosinophilic fibrillary background (neurophil or neuritic processes of primitive neuroblasts) is indicative of a neural origin.
- Homer Wright pseudorosettes (tumour cells arranged around a central space filled with their fibrillar extensions) may be seen.

Clinical Features

- Usually present with a large abdominal mass, fever and weight loss.
- Metastases may cause hepatomegaly, ascites and bone pain.
- In neonates, disseminated neuroblastomas may present with multiple cutaneous metastases and deep blue discolouration of the skin **(blueberry muffin baby)**.
- About 90% tumours produce catecholamines and are associated with elevated levels of catecholamine metabolites like vanillylmandelic acid (VMA) and homovanillic acid (HVA) in the urine.

The important prognostic features of neuroblastoma are enlisted in Table 8.4.

TABLE 8.4. Prognostic indicators of neuroblastoma

Features	Good prognosis	Bad prognosis
Age	<18 months	>18 months
Stage	1, 2a, 2b, 4s	III or IV
Ploidy	Hyperdiploid/near triploid	Near diploid
1p deletion and *N-myc* amplification	Absent	Present
Expression of TrkA (high affinity growth receptor) indicating differentiation towards sympathetic ganglia lineage	Present	Absent
Expression of TrkB	Absent	Present
Mutations of neuritogenesis genes	Absent	Present
Morphology	Presence of Schwannian stroma and gangliocytic differentiation	Absence of Schwannian stroma and gangliocytic differentiation
Mitosis-karyorrhexis index	<200/5000 cells	>200/5000 cells

Staging

- Stage 1: Localized tumour that is completely surgically removed at diagnosis.
- Stage 2A: Unilateral tumour with incomplete gross resection; identifiable, ipsilateral and contralateral lymph nodes negative for tumour.
- Stage 2B: Localized tumour with or without complete gross resection; representative ipsilateral nonadherent lymph nodes positive for tumour; enlarged contralateral lymph nodes are negative for tumour microscopically.
- Stage 3: Unresectable unilateral tumour infiltrating across the midline with or without lymph nodes involvement or localized unilateral tumour with contralateral regional lymph nodes involvement.
- Stage 4: Any primary tumour with dissemination to distant lymph nodes, bone, bone marrow, liver, skin or other organs except defined for stage 4s.
- Stage 4s: Localized primary tumour (as defined for stages 1, 2A or 2B) with dissemination limited to skin, liver and/or bone marrow (less than 10% of nucleated cells are constituted by the neoplastic cells; more than 10% involvement of bone marrow is considered stage 4).

Q. Describe the clinicopathological features of retinoblastoma.

Ans. Retinoblastoma is the most common malignant tumour of the eye in childhood.

- It frequently occurs as a congenital tumour.
- About 60–70% of the tumours are associated with a germline mutation in the RB1 gene and are inherited; 30–40% of the tumours develop sporadically and have a somatic RB1 gene mutation.
- Occurs in both familial (may be multifocal and bilateral) and sporadic patterns.
- May undergo spontaneous regression.
- Patients have a high incidence of secondary tumours. (Patients with familial retinoblastoma are at increased risk of developing osteosarcoma and other soft-tissue sarcomas.)
- Most cases are diagnosed before the age of 4 years.

Clinical Features

- Median age at presentation is 2 years.
- Presenting findings include poor vision, strabismus, a whitish hue to the pupil **(cat's eye reflex)** and pain in the eye.

Morphology

- Arises from neuroepithelial cells.
- Nodular, often with satellite lesions.
- Composed of small round cells with hyperchromatic nuclei and scant cytoplasm (resembling retinoblasts).
- True rosettes called ***Flexner–Wintersteiner rosettes*** (clusters of cuboidal or short columnar cells arranged around a central lumen, which seems to have a limiting membrane resembling the external limiting membrane of the retina) are present and are unlike the pseudorosettes of neuroblastoma, which lack a true lumen.
- Tumour cells spread along the optic nerve or subarachnoid space.
- Distant metastases is seen in CNS, skull, distal bones and lymph nodes.

Prognosis

Untreated the tumour is fatal, but with enucleation, chemotherapy and radiotherapy, survival rates are usually good.

9

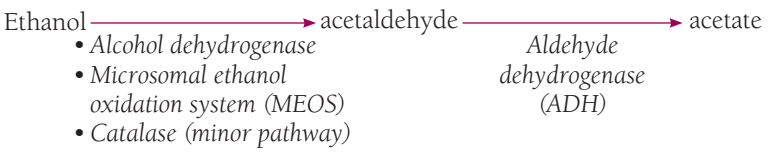
Environmental and Nutritional Pathology

Q. Write briefly about environmental toxins.

Ans. Environmental pathology can be induced due to personal or occupational exposure to toxins. Environmental toxins are the contaminants released into natural environment that cause instability in the ecosystem and harm to the living organisms inhabiting it. Xenobiotics are environmental toxins that may be absorbed into the body by inhalation, ingestion or through the skin and mucosal surfaces. They include naturally occurring toxic substances like carbon dioxide, hydrogen sulphide and volcanic gases, or industrial waste like heavy metals (lead, cadmium, etc.), pesticides used in agriculture and industry, chlorine and other disinfectants and wood preservatives. When the ability of our bodies to defend these toxins is exceeded or when our bodies fail to break down or remove these toxins, serious damage may ensue. Self-induced exposure or addictions to certain substances can result in health effects. These include alcohol, tobacco and drugs like opioid narcotics, cannabinoids and sedative-hypnotics.

Alcohol

- *Ethanol* is mainly absorbed in the stomach and small intestine; depending on the blood levels, it is then distributed to all tissues and fluids of the body.
- Drunk driving in most states is defined as a concentration of 80 mg/dL in the blood; inebriation generally results at a blood alcohol level of 200 mg/dL and coma, death and respiratory arrest usually occur at levels of 300–400 mg/dL.
- Habitual drinkers can tolerate blood alcohol levels up to 700 mg/dL. This metabolic tolerance is attributed to an increased induction of the cytochrome P-450 xenobiotic-metabolizing enzyme CYP2E1, which accelerates the metabolism of ethanol as well as that of other drugs and chemicals like cocaine and acetaminophen.
- *Methanol* is metabolized to formaldehyde and formic acid, resulting in metabolic acidosis, dizziness, vomiting, blurred vision or blindness and respiratory depression.
- Ethanol in blood is transformed to acetaldehyde in the liver by three enzyme systems.



Adverse Effects of Alcohol

1. **Acute effects:** Acute alcohol intake exerts its effects mainly on CNS, but can also induce hepatic and gastric damage. The following are chief manifestations of acute alcoholic toxicity:
   (a) CNS: Disordered cortical, motor and intellectual behaviour followed by CNS depression
   (b) Liver: Acute alcoholic hepatitis (manifests with fever, right hypochondrial tenderness, jaundice and histology of the liver shows focal hepatocyte necrosis, Mallory hyaline, neutrophilic infiltrate and fat accumulation)
   (c) Stomach: Acute gastritis or ulceration

2. **Chronic effects:** Chronic alcohol intake has deleterious effects on specific organs as described:
   (a) Liver: Fatty change, hepatitis, cirrhosis, portal hypertension or hepatocellular carcinoma are possible effects (alcohol oxidation by ADH causes reduction in nicotinamide adenine dinucleotide or NAD levels as ADH reduces NAD to NADH. NAD is required for fatty acid oxidation in liver and conversion of lactate into pyruvate. Its deficiency, therefore, causes accumulation of fat in the liver of alcoholics).
   (b) Nervous system: Thiamine deficiency is common in alcoholics and is known to induce degeneration of nerve cells, reactive gliosis and atrophy of cerebellum and peripheral nerves. Two syndromes are closely associated with chronic alcohol intake, namely, **Wernicke syndrome**, which presents with ataxia, disturbed cognition, ophthalmoplegia, nystagmus and **Korsakoff syndrome**, which is believed to result from a combination of alcohol toxicity, poor nutrition and thiamine deficiency, and manifests with severe memory loss.
   (c) CVS: Chronic alcohol intake causes injury to myocardium leading to *dilated congestive cardiomyopathy,* which is thought to be due to direct toxicity rather than thiamine deficiency. High blood alcohol levels have a vasopressor effect due to release of catecholamines, which may induce hypertension. Heavy consumption of alcohol also leads to decreased levels of high-density lipoproteins (HDL) and contributes to *coronary artery disease*.
   (d) GIT: Alcohol can cause massive bleeding from gastritis/gastric ulcer or oesophageal varices and acute or chronic pancreatitis. It is also associated with increased risk of cancer *of the oral cavity,* and *oesophagus*. Ethanol is not a direct-acting carcinogen; but one of its metabolites, acetaldehyde, may act as a tumour promoter. Ethanol inhibits detoxification of chemical carcinogens such as nitrosamines, which have been associated with tumours of the upper gastrointestinal tract.
   (e) Reproductive system: Heavy long-term consumption of alcohol is known to cause testicular atrophy in men and reduced fertility and spontaneous abortions in women.
   (f) Skeletal muscle: Alcohol causes rhabdomyolysis, which, in turn, leads to muscle weakness and pain.
   (g) Ethanol is a substantial source of energy; therefore, chronic alcoholism commonly leads to malnutrition and deficiencies.
   (h) Alcohol intake during pregnancy can induce **fetal alcohol syndrome**, which manifests in infants as microcephaly, growth retardation and facial abnormalities; older children may show a reduction in mental functions.

Smoking

Tobacco is the most frequent exogenous cause of human cancer. Main contributor is cigarette smoking, but pipes, snuff and tobacco chewing are also harmful. Smoking associated cancers include cancer of the larynx, lung, oesophagus, pancreas, urinary bladder and oral cavity. The important toxic chemicals present in tobacco smoke are enlisted in Table 9.1.

TABLE 9.1. Toxic chemicals in tobacco smoke

Chemicals	Effects
• Tar • Polycyclic aromatic hydrocarbons • Nitrosamines	Carcinogenesis
• Nicotine	Ganglionic stimulation and depression and tumour promotion
• Phenol	Tumour promotion
• Formaldehyde • Nitrogen oxide	Irritation and toxicity to respiratory mucosa
• Carbon monoxide	Reduced oxygen transport

Other Effects of Smoking

- Ischaemic heart disease (due to accelerated atherosclerosis, increased platelet aggregation and impaired lung function, which causes reduced myocardial oxygen supply)
- Peptic ulcer disease
- Cerebrovascular accident (CVA)
- Chronic respiratory disease (exacerbates bronchitis, asthma and pneumoconiosis)
- Increased incidence of low birthweight, prematurity and spontaneous abortion as a consequence of maternal smoking

Air Pollution

1. Outdoor: problem of industrialized countries. Following are the major sources:
   (a) Combustion of fossil fuels from vehicles, power plants and factories
   (b) Photochemical reactions (oxides of nitrogen and hydrocarbons interact to produce 'ozone' which causes decreased lung function, increased airway/lung inflammation and decreased exercise capacity)
   (c) Power plant emissions (sulphur dioxide which causes decreased lung function)
   (d) Waste from incinerators/industry/smelters (acid aerosols, organic compounds and particles, which damage the mucociliary apparatus, decrease lung function and increase respiratory infections)
   (e) Automotive engines, industries using fossil fuels and home heating with oil and cigarette smoke (carbon monoxide or CO which is a nonirritant, colourless and odourless gas produced by imperfect combustion of carbonaceous material)
2. Indoor: caused by
   (a) CO: Can cause acute poisoning
   (b) NO_2: Predisposes to respiratory infections
   (c) Wood smoke: Contains oxides of nitrogen and carbon particulates which are irritants and predispose to lung infections
   (d) Formaldehyde: Causes eye and nose irritation and asthma
   (e) Radon: Radioactive gas derived from uranium widely present in soil and homes; can cause lung cancer in uranium miners
   (f) Asbestos fibres: Occupational exposure can produce lung cancer and mesotheliomas
   (g) Mineral fibres: Used in maintenance and construction; may cause skin and airway irritation
   (h) Bioaerosols: Include microbiologic agents which cause infections such as Legionnaires disease, viral agents, common cold as well as allergens derived from pet dander, dust mites, fungi and moulds that cause allergic rhinitis/asthma

Carbon Monoxide

- Important cause of accidental death due to oxygen deprivation (haemoglobin has 200 times higher affinity for CO than for O_2; besides, carboxyhaemoglobin interferes with the release of oxygen from oxyhaemoglobin causing further tissue hypoxia)
- Diagnosis of CO poisoning confirmed by measuring carboxyhaemoglobin levels
- CO poisoning presents in two ways:
  - *Acute CO poisoning*
    - Cherry red skin and mucous membrane
    - Petechial haemorrhages
    - Hypoxic injury to brain, liver and renal tubules
  - Chronic CO poisoning
    - Diffuse neuronal loss and focal cerebral demyelination (CNS disturbances, hearing loss, blindness and paralysis)
    - May cause glomerulopathy and acute tubular necrosis (ATN)

Mercury

- ***Source*:** Industrial contamination of ocean (from bacteria to fish to humans) and paints
- ***Effects*:** CNS disturbances, hearing loss, blindness, spasticity, paralysis and glomerulopathy

Cyanide

- ***Sources*:** Combustion of wool, silk and plastic upholstery
- ***Effects*:** Hypoxic injury to brain, liver and kidney

Mushroom Poisoning

- ***Sources*:** *Amanita phalloides* and *Amanita muscaria*
- ***Effects*:** Vomiting, abdominal cramps, CNS changes, renal and hepatic necrosis

Insecticides

- ***Sources*:** Chlorinated hydrocarbons, DDT, chlordane and organophosphates
- ***Effects*:** Hyperexcitability, muscle twitching to paralysis, cardiac arrhythmias, delirium, convulsions and coma

Methanol

- ***Source*:** Organic solvents
- ***Effects*:** Toxic necrosis of retinal ganglion cells with blindness

Q. Write briefly about lead toxicity.

Ans. Lead exposure occurs through contaminated air, water and food.

Sources

- House paints, gasoline, mines, foundries, batteries, automatic exhaust, urban soil and spray paints
- Most of the absorbed lead is taken up by bone and teeth.

Effects of Lead Poisoning

1. ***Bones*:** Radiodense deposits in epiphyses (excess lead interferes with vitamin D metabolism and calcium homoeostasis thereby interfering with normal remodelling of calcified cartilage and primary bone trabeculae in the epiphyses of children)
2. ***Nervous system*:** Excess lead causes **neurological effects** in adults and children (encephalopathies, demyelination, peripheral neuropathies, low intellectual capacity, hyperactivity, poor organizational skills, headache and memory loss)
3. ***Gingiva*:** Lead line (lead stimulates hyperpigmentation of the gums)
4. ***Blood*:** Lead has high affinity for sulphydryl groups and interferes with enzymes involved in haem synthesis; iron incorporation into haem is impaired leading to microcytic hypochromic anaemia and basophilic stippling. The levels of erythrocyte protoporphyrin are increased.
5. ***Kidney*:** Chronic tubulointerstitial disease (excretion of lead occurs via the kidney)
6. ***GIT*:** Abdominal pain or colics

Q. Write briefly about the effects of climate change on human health.

Ans. During the preceding 1000 years, maximum warming of earth has taken place in the last 50 years. Rising levels of CO_2, methane and ozone (greenhouse gases) along with water vapour lead to increased absorption and re-emission of infrared energy which radiates from the surface of the earth and normally lost into space. This event raises the global temperature (greenhouse effect).

- Increase in global temperature increases the surface heat absorption leading to:
  a) Loss of ice and snow
  b) Increased content of atmospheric water vapour due to increased evaporation from water bodies

c) Increased release of CO_2 and methane from organic matter in thawing ice (such as arctic)
d) Decreased removal of CO_2 by diatoms due to their reduced growth; increased CO_2 increases the acidity of oceans which in turn disrupts the marine ecosystem
e) Increased heat energy in oceans induces variability in the weather events casing floods, droughts and storms

- Climate change brings about **cardiovascular, cerebrovascular** and **respiratory diseases** (owing to increased temperature and air pollution); **increased incidence of food- and water-borne diseases** (contamination due to floods and disruption of clean water supply); **increased incidence of vector-borne infections** (due to increased temperature) and **malnutrition** (weather changes harm the crop production).

Q. Write briefly about the biological effects and mechanism of action of ionizing radiation.

Ans. Radiation describes a process in which high-energy particles or waves travel through a medium or space. There are two distinct types of radiation:

- **Ionizing radiation:** The word *radiation* is commonly used in reference to ionizing radiation only (ie, having sufficient energy to ionize an atom). This occurs when an electron is stripped (or 'knocked out') from an electron shell, and leaves the atom with a net positive charge, eg, alpha particles (consist of two neutrons and two protons), beta particles (consist of energized electrons), gamma rays (consist of photons with a frequency of greater than 10^{19} Hz) and X-rays (electromagnetic waves with a wavelength smaller than about 10 nm).
- **Nonionizing radiation:** Energy radiating (ie, travelling outward in straight lines in all directions) from its source. The energy of nonionizing radiation is less and instead of producing charged ions when passing through matter, there is only sufficient energy to change the rotational, vibrational or electronic valence configurations of molecules and atoms, eg, UV rays, infrared waves, microwaves and sound waves.

Radiation Units

1. **Curie (Ci):** The amount of radiation emitted by a source (one Ci = 3.7×10^{10} disintegrations per second of a radionuclide/radioisotope).
2. **Gray (Gy):** The energy absorbed by the target tissue per unit mass; corresponds to the absorption of 10^4 erg/gm of tissue and is equivalent to exposure of tissues to 100 RAD (radiation absorbed dose). The unit 'cGy' (centigray) terminology has replaced 'R' (rads).
3. **Sievert (Sv):** Has replaced a term called 'rem'. Sv quantifies a unit of equivalent dose that depends on the biological rather than the physical effects of radiation (measures the relative biological effectiveness of the radiation).

Morphological Effects of Radiation

- Deletions, breaks, translocations and fragmentation of chromosomes
- Disorderly mitotic spindles, polyploidy and aneuploidy
- Nuclear swelling/condensation/clumping of chromatin
- Breakdown of nuclear membrane
- Apoptosis
- Giant cells, pleomorphic nuclei, cytoplasmic swelling, degeneration of mitochondria and endoplasmic reticulum and defects in plasma membrane
- Vascular changes, eg, endothelial swelling and proliferation with hyalinization of vessel wall

Hazards of Radiation

These are caused by the whole body irradiation having short wavelength and high frequency. It may be electromagnetic waves like X-rays or particulate materials like α particles, β particles, electrons, protons, neutrons, mesons and deuterons. The clinical manifestations depend on the dose and duration of exposure to such radiation (Table 9.2).

TABLE 9.2. Effects of whole body irradiation

	0–1 Sv	1–2 Sv	2–10 Sv	10–20 Sv	>50 Sv
Major site of injury	–	Lymphocytes	Bone marrow	Small bowel	CNS
Clinical presentation	–	Neutropenia and lymphopenia	Leukopenia, haemorrhage, alopecia and vomiting	Nausea, vomiting, diarrhoea and electrolyte imbalance	Nausea, vomiting, ataxia, convulsion, coma
Duration	–	1 day to 1 week	2–7 weeks	5–15 days	1–6 h
Mortality	–	–	+/−	100%	100%

Chronic Effects of Radiation

Result from damage to the genetic material in dividing cells, causing abnormalities of cell growth, such as cancer. Damage to reproductive cells has been shown to lead to birth defects. External radiation therapy for cancer may cause nausea, vomiting and loss of appetite, skin changes including hair loss, redness, peeling, sores, thinning of the skin, dilated blood vessels just beneath the skin's surface (spider veins) and skin cancer. Radiation to the lungs can cause radiation pneumonitis and fibrosis. Radiation to GIT may cause ulcers, fibrosis, strictures and cancer. Testes and ovaries show tubular atrophy and stromal fibrosis, respectively, and CNS may show necrosis, gliosis and cancer.

Q. Write briefly about exogenous oestrogens and their effects.

Ans. Oestrogen is extensively used in:

1. Menopausal hormone therapy (MHT):
   (a) Given to postmenopausal women to prevent progression of osteoporosis, distressing menopausal symptoms like hot flushes and reduce the likelihood of myocardial infarction (protective role of MHT in myocardial infarction has been demonstrated only in women under the age of 60 years; no protection in women who start MHT after 60 years is seen).
   (b) Unopposed oestrogen therapy increases the risk of **endometrial cancer;** risk is reduced or eliminated when progestins are added.
   (c) MHT may cause an increase in risk of **breast carcinoma** after a median time of 5–6 years (risk of breast carcinoma is marginally reduced with oestrogen-only therapy in females who have undergone hysterectomy).
   (d) MHT increases the risk of **venous thromboembolism (deep vein thrombosis, pulmonary embolism and stroke)** by about twofold.
2. Oral contraceptives (OCs)
   (a) Usually contain a synthetic oestradiol and a variable amount of progestin; few preparations contain progesterone only. OCs inhibit ovulation and prevent implantation.
   (b) Current prevailing opinion is that OCs do not cause an increase in breast cancer risk.
   (c) OCs have a protective role in endometrial and ovarian cancer.
   (d) May increase risk of cervical carcinoma in women infected with HPV virus (increased risk may be due to increased sexual activity).
   (e) Increase risk of thromboembolism. OCs induce a hypercoagulable state as they increase hepatic synthesis of coagulation factors.
   (f) Risk of cardiovascular disease increases in women over 35 years who are smokers.
   (g) Well-defined association between the use of OCs and hepatic adenoma.

Q. Define protein energy malnutrition (PEM).

Ans. Inadequate consumption of protein and/or energy resulting in a range of clinical syndromes, namely, **kwashiorkor** and **marasmus**.

Q. Differentiate between kwashiorkor and marasmus.

Ans. Differences between kwashiorkor and marasmus are enlisted in Table 9.3.

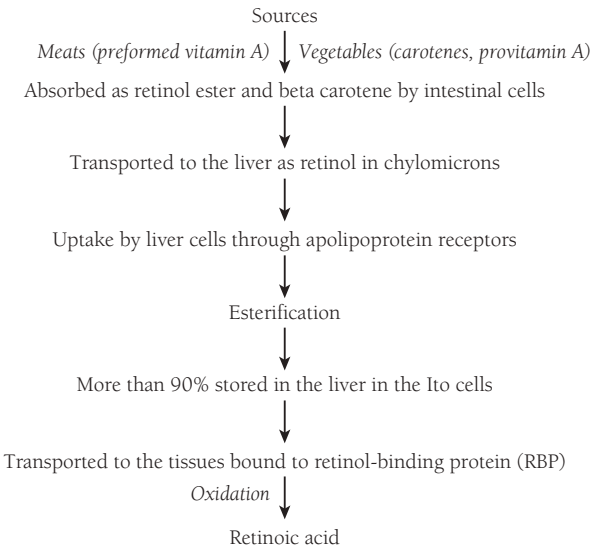
TABLE 9.3. Differences between kwashiorkor and marasmus

Features	Kwashiorkor	Marasmus
Definition	Protein deficiency with adequate calorie intake	Starvation with a lack of overall calories
Age	<3 years	0–2 years
Clinical features		
Skeletal muscle	Relatively spared	Catabolized, loss of muscle mass
Subcutaneous fat	Spared	Mobilized for energy
Liver protein stores	Markedly deprived/reduced—sometimes life-threatening	Depleted only marginally
Serum protein levels	Markedly decreased	Normal/slightly decreased
Oedema	Present; may be generalized or dependent	Absent
Extremities	Oedematous	Patient looks emaciated; head appears too large as compared to body
Growth/mental retardation	Present but much less	Present; more severe
Weight loss	60–80% of normal weight for the age and sex	Falls below the 60% of normal range
Skin lesions	'**Flaky paint**' appearance (alternating zone of hyperpigmentation, desquamation and hypo pigmentation)	Not generally seen
Hair changes	Loss of colour, alternating bands of pale and darker hair **(flag sign)**, excessive hair fall	Not generally seen
Hepatomegaly	Presents with fatty change	Not seen
Appetite	Lost; patient is apathetic, listless	Hungry and alert
Small bowel	Decrease in mitotic index in crypts, associated with mucosal atrophy and loss of villi	Rarely seen
Thymic and lymphoid atrophy	More marked	Less marked
Immune deficiency/ recurrent infections	Present, lesser	Immune deficiency (mostly T cell) present, prone to recurrent infection

Q. Write briefly about the metabolism of vitamin A.

Ans. Vitamin A (fat-soluble vitamin) is the generic term used for a group of related compounds namely, **retinal, retinol and retinoic acid.**

- **Retinol (an alcohol):** Chemical name for vitamin A is the transport form; storage form is a retinol ester.
- **Retinal (an aldehyde):** Can be converted by the body to retinoic acid.
- **Retinoids:** Refers to both natural and synthetic chemicals that are structurally related to vitamin A but may not have similar activity.
- **Animal sources:** Liver, fish, eggs, milk and butter are important dietary sources of preformed vitamin A.
- **Yellow and green leafy vegetables (spinach, carrots and squash)** supply large amounts of **beta-carotene and other carotenoids** that can be converted by the body into retinol and are referred to as **provitamin A carotenoids (**Flowchart 9.1**).**



Note: The uptake of retinol in the peripheral tissue is dependent on cell surface receptors that are specific for RBP rather than retinol.

FLOWCHART 9.1. Metabolism of vitamin A.

Functions

1. Visual process: The retina is located at back of the eye. When light passes through the lens, it is sensed by the retina and converted to a nerve impulse for interpretation by the brain.
   (a) *Retinol* is transported to the retina via circulation and accumulates in retinal pigment epithelial cells; retinol is esterified to form a *retinyl* ester, which can be stored.
   (b) When needed, retinyl esters are broken apart (hydrolysed) and isomerized to form 11-*cis-retinol,* which can be oxidized to form 11-*cis-retinal.*
   (c) 11-*cis*-retinal can be shuttled across to rod cells, where it binds to a protein called opsin to form the visual pigment, *rhodopsin (also known as visual purple).*
   (d) Rod cells with rhodopsin can detect very small amounts of light, making them important for night vision. Absorption of a photon of light catalyses isomerization of 11-*cis-retinal* to *all-trans-retinal,* and results in its release. This isomerization triggers a cascade of events, leading to generation of an electrical signal to optic nerve.
   (e) Once released, *all-trans-retinal is converted to all-trans-retinol,* which can be transported across retinal epithelial cell, thereby completing the visual cycle.
   (f) Inadequate retinol available to the retina results in impaired dark adaptation, known as 'night blindness'.
2. Role in orderly differentiation of mucous-secreting epithelium:
   (a) *All-trans-retinoic acid (ATRA)* exerts its effect by binding to retinoic acid receptor (*RAR*), which in turn is associated with nuclear receptors for 9-*cis*-retinoic acid (*RXR*) *forming RAR/RXR heterodimers*. These bind to promoter regions of multiple genes encoding for growth factors and tumour suppressor genes.
   (b) *ATRA* induces temporary remission of acute promyelocytic leukaemia and the *retinoic acid isomer,* 13-*cis retinoic acid,* has been used in the treatment of neuroblastoma.
3. Role in host resistance to infections:
   (a) Stimulates the immune system (probably humoral immunity)
   (b) Maintains and restores integrity of mucosa

Q. Enlist the manifestations of vitamin A deficiency.

Ans. Manifestations of vitamin A deficiency

- First symptom of vitamin A deficiency is decreased night vision (vision in dim light).
- Ocular changes due to vitamin A deficiency are collectively known as *xerophthalmia (dry eye)*.
- First change is dryness of the conjunctivae, wherein, normal lacrimal and mucous-secreting epithelium is replaced by keratinized epithelium.
- This is followed by accumulation of keratin debris as small opaque plaques called *Bitot spots*. There is erosion of surface of cornea, inducing formation of *corneal ulcers*, which may later lead to *softening and destruction of cornea (keratomalacia)* and *blindness*.
- The epithelium lining upper respiratory passage and urinary tract is replaced by keratinized squamous epithelium (*squamous metaplasia*).
- Loss of mucociliary apparatus predisposes to secondary bacterial infections.
- Desquamation of keratinous debris in the urinary tract leads to renal and urinary bladder stones.
- Hyperplasia and hyperkeratinization of epidermis results in plugging of ducts of adnexal glands producing *follicular* or *papular dermatosis*.

Q. Write briefly about the metabolism of vitamin D.

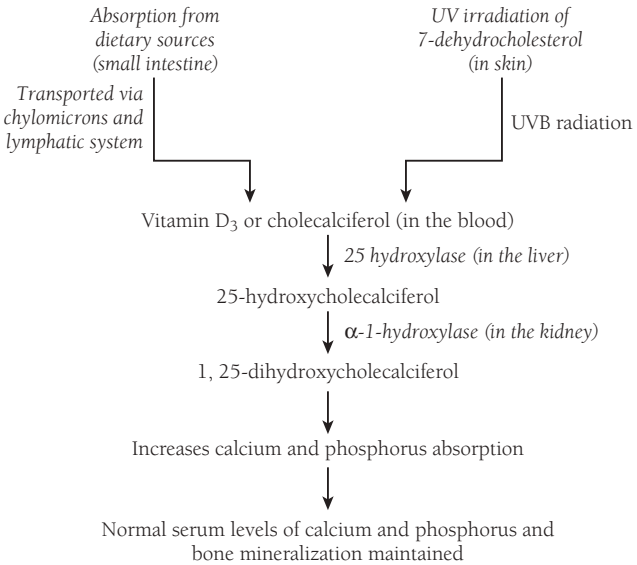
Ans. Vitamin D is necessary for the formation, growth and repair of bones. It also enhances immune function and improves muscle strength. Requirement for vitamin D increases as people age. It is stored mainly in the liver.

Forms of Vitamin D

The following are the two forms of vitamin D important for nutrition:

1. **Vitamin D_2 (ergocalciferol):** This form is synthesized from plants and yeast precursors. It is also the form used in very high dose supplements.
2. **Vitamin D_3 (cholecalciferol):** This form is the most active form of vitamin D. It is formed in the skin when the skin is exposed to direct sunlight. The most common food source is fortified foods, mainly cereals and dairy products. Vitamin D_3 is also present in fish liver oils. Human breast milk contains only small amounts of vitamin D_3.

Vitamin D_2 and D_3 are not active in the body. Both forms must be metabolized by the liver and kidneys into an active form called *calcitriol* (Flowchart 9.2).



FLOWCHART 9.2. Metabolism of vitamin D.

Q. Enumerate the factors that predispose to rickets and osteomalacia.

Ans. Factors predisposing to rickets and osteomalacia are

- Inadequate synthesis or dietary deficiency of vitamin D
  - Inadequate exposure to sunlight
  - Limited dietary intake of fortified foods
  - Poor maternal nutrition during pregnancy and breastfeeding
- Decreased absorption of vitamin D (fat-soluble vitamin)
  - Cholestatic liver disease
  - Biliary tract obstruction
  - Pancreatic insufficiency
  - Diseases of small intestine
- Deranged vitamin D metabolism
  - Impaired synthesis of 25-hydroxy vitamin D
  - Increased degradation of vitamin D and 25-hydroxy vitamin D
  - Decreased synthesis of 1,25-dihydroxy vitamin D
  - Resistance to action of 1,25-dihydroxy vitamin D

Q. Enlist the manifestations of vitamin D deficiency.

Ans. The following are the salient features of vitamin D deficiency:

- The most common cause is inadequate exposure to sunlight. Thus, vitamin D deficiency occurs mainly among people who do not spend much time outdoors.
- Because breast milk contains only small amounts of vitamin D, breastfed infants are at risk of rickets.
- In malabsorption disorders, patients cannot absorb vitamin D because it is a fat-soluble vitamin, which is normally absorbed with fats in the small intestine.
- The body may not be able to convert vitamin D to an active form. Certain kidney and liver disorders and several rare hereditary disorders interfere with this conversion, as do certain drugs, such as some anticonvulsants and rifampin.

Manifestations of Vitamin D Deficiency

- Manifests with rickets in children and osteomalacia in adults.
- In young infants who have rickets, the entire skull may be soft. Older infants may be slow to sit and crawl, and the spaces between the skull bones (fontanelles) may be slow to close.
- In children aged 1–4 years, bone growth may be abnormal, causing an abnormal curve in the spine and **bow legs or knock-knees**. These children may be slow to walk.
- For older children and adolescents, walking is painful. The pelvic bones may flatten, narrowing the birth canal in adolescent girls.
- Deformities of skeleton like **craniotabes** (parietal bones buckle inwards by pressure; with the release of pressure, elastic recoil snaps the bone back into its original position), **frontal bossing, squared appearance of frontal head** (due to excess of osteoid), **rachitic rosary** (overgrowth of cartilage or osteoid tissue at the costochondral junction), **pigeon chest deformity** (weakened metaphyseal areas of the ribs are subject to the pull of respiratory muscles and thus bend inward, creating anterior protrusion of the sternum) and **Harrison groove** (created by the inward pull at the margin of the diaphragm) are also seen.
- In adults, the spine is affected (**lumbar lordosis**), pelvis and leg bones weaken. Affected areas may be painful to touch, and fractures may occur.

Q. Write briefly about the sources, functions and deficiency of vitamin C.

Ans. The sources of vitamin C includes Milk, fish, liver, fruits and vegetables; cannot be synthesized endogenously, thus humans are dependent on dietary intake.

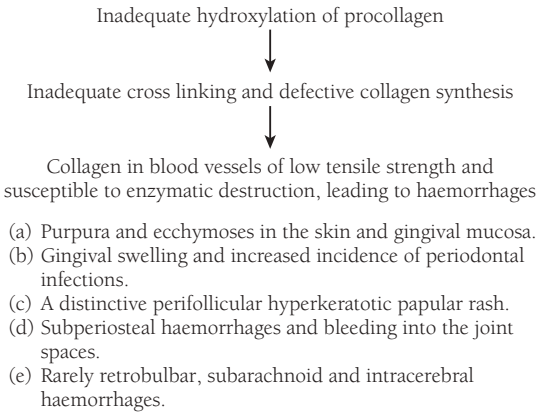
Functions of Vitamin C

- **Role in collagen synthesis:**
  - Accelerates hydroxylation and amidation reactions
  - Activates prolyl and lysyl hydroxylases for hydroxylation of procollagen (hydroxylation important for a stable helical structure and cross linking)
- **Antioxidant action:**
  - Scavenges free radicals and regenerates the antioxidant form of vitamin E; vitamins C and E act in concert to reduce atherosclerosis by reducing the oxidation of LDL

Deficiency of Vitamin C (Scurvy)

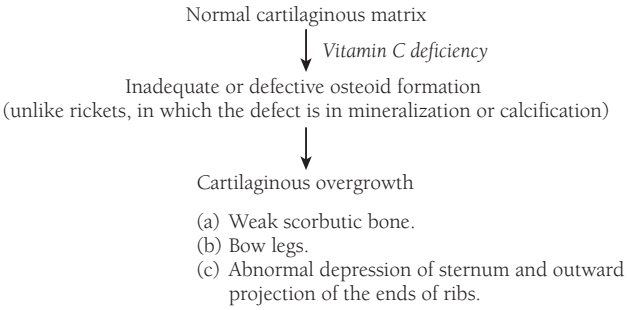
Clinical manifestations of vitamin C deficiency are:

1. **Haemorrhage (Flowchart 9.3)**



FLOWCHART 9.3. Pathogenesis of haemorrhage in vitamin C deficiency.

2. **Skeletal changes (Flowchart 9.4):**



FLOWCHART 9.4. Pathogenesis of skeletal changes in vitamin C deficiency.

3. **Delayed wound healing**
4. **Anaemia due to blood loss**

Q. Write briefly about vitamin E deficiency.

Ans. Vitamin E belongs to a group of eight closely related fat-soluble compounds (four tocopherols and four tocotrienols) of which α-tocopherol is the most active.

- Sources are vegetables, nuts, grains and their oils, dairy products, fish and meat
- Absorption requires normal biliary and pancreatic function
- Transported in the blood in chylomicrons
- Stored mainly in the fat depots; also in minor amounts in the liver and muscle.
- Functions as an antioxidant (terminates free radical generated lipid peroxidation reactions and prevents damage to the cellular and subcellular membranes)
- Pathologic changes due to vitamin E deficiency are characteristically seen in the nervous system (degeneration of axons in the posterior columns of the spinal cord and loss of neurons in the dorsal root ganglia). Vitamin E–deficient RBCs are more susceptible to oxidative damage and have a shorter half-life
- Manifestations include depressed tendon reflexes, ataxia, dysarthria, loss of position and vibration sense, muscle weakness, impaired vision and disorders of eye movement

Q. Write briefly about vitamin K deficiency.

Ans. Vitamin K is a cofactor for a liver microsomal carboxylase, which carboxylates the glutamyl residues in certain proteins to carboxyglutamates, eg, clotting factors like prothrombin, factors VII, IX and X. Carboxylation provides a calcium-binding site to allow calcium-dependent interactions of the clotting factors.

- Activation of **proteins C and S** also requires vitamin K-dependent carboxylation.
- Vitamin K–dependent carboxylation of **osteocalcin** (a protein secreted by osteoblasts) increases calcium and osteocalcin interaction and thereby favours bone calcification.
- Endogenous intestinal bacteria synthesize the vitamin, therefore, it is required in very small amounts in the diet.
- Active reduced form is converted to an epoxide after vitamin K reacts with its substrate; the epoxide reduced back by a liver epoxide reductase.
- Deficiency is seen in
  (a) Malabsorption syndromes (vitamin K is a fat-soluble vitamin)
  (b) Ingestion of broad spectrum antibiotics (which destroy the gut flora)
  (c) Neonatal period when the intestinal flora is not well developed, liver reserves are small and breast milk is low on vitamin K
- Deficiency manifests as bleeding diathesis (haematomas, haematuria, melena, ecchymoses and bleeding from the gums).

Q. Write briefly about thiamine deficiency.

Ans. Thiamine plays an important role in helping the body metabolize carbohydrates and fats to produce energy.

- Absorption of thiamine in the gut is followed by its phosphorylation resulting in formation of thiamine pyrophosphate, which has three important functions:
  - It regulates the oxidative decarboxylation of α-keto acids, responsible for synthesis of adenosine triphosphate
  - It is a cofactor for transketolase in pentose phosphate pathway
  - It maintains the integrity of neural membrane and ensures normal nerve conduction
- Thiamine is essential for normal growth and development, and helps to maintain proper functioning of the heart, nervous and digestive systems.
- It is water-soluble and cannot be stored in the body; however, once absorbed, the vitamin concentrates in muscle tissue.

- **Sources** include green peas, spinach, liver, beef, pork, beans, nuts, bananas, whole grains, unpolished rice and legumes.
- **Deficiency** manifests as **Wernicke–Korsakoff syndromes** or **dry** and **wet beriberi**, and usually results from malnutrition, alcoholism, diets high in thiaminase-rich foods (raw freshwater fish, raw shellfish and ferns) and antithiamine factors (tea and coffee), debilitating illness, consumption of polished rice and protracted diarrhoea.
- **Dry beriberi** causes wasting and partial paralysis resulting from damaged peripheral nerves. It is also associated with tingling or loss of feeling (sensation) in hands and feet, mental confusion, speech difficulties and involuntary eye movements (nystagmus). It is thought to result from degeneration of myelin.
- **Wet beriberi** mainly affects the heart; it causes vasodilation, peripheral oedema, paroxysmal nocturnal dyspnoea, increased heart rate and eventually heart failure. The chronic form of wet beriberi consists of three stages. In the first stage, peripheral vasodilatation occurs, leading to a high-cardiac-output state. This leads to salt and water retention mediated through renin–angiotensin–aldosterone system in the kidneys. As the vasodilation progresses, the kidneys detect a relative loss of volume and respond by conserving salt. With salt retention, fluid is also absorbed into the circulatory system. The resulting fluid overload leads to oedema of dependent extremities. By the time significant oedema occurs, the heart has been exposed to a severely high workload in order to pump required cardiac output needed to satisfy end-organ requirements. This causes parts of the heart muscle to undergo overuse injury.
- A more rapid form of wet beriberi is termed **acute fulminant cardiovascular beriberi** or **Shoshin beriberi**. In this form, oedema may not be present. Instead, cyanosis of hands and feet, tachycardia, distended neck veins, restlessness and anxiety occur. It is because of damage to the heart muscle and its inability to cope with the demands of the body. Treatment with thiamine causes low-output cardiac failure because systemic vasoconstriction is reinstated before the heart muscle recovers.

Q. Write briefly about riboflavin deficiency.

Ans. Riboflavin is a yellow or yellow-orange coloured vitamin which can be used as a food colouring.

- Large quantities of riboflavin are often included in multivitamins. The excess is excreted in the urine, causing the urine to be coloured bright yellow.
- **Deficiency** of riboflavin can be **primary**, which is diet related, or **secondary**, which may be a result of conditions that affect absorption in the intestine, the body not being able to use the vitamin, or an increase in the excretion of the vitamin from the body.
- Riboflavin deficiency manifests as cracked and red lips, inflammation of the lining of mouth and tongue, mouth ulcers, cracks at the corners of the mouth (**angular cheilitis**) and a sore throat.
- Deficiency may also cause dry and scaly skin, scrotal dermatitis, fluid in the mucous membranes and iron-deficiency anaemia. The eyes may become bloodshot, itchy, watery and sensitive to bright light (**photophobia**).

Q. Write briefly about niacin deficiency.

Ans. Niacin (Vitamin B_4) refers to both nicotinic acid and its amide derivative nicotinamide (niacinamide). Both of the above are required for formation of coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP).

- The coenzymes NAD and NADP are required for many biological oxidation–reduction (redox) reactions responsible for energy generation in tissues by the biochemical degradation of carbohydrates, fats and proteins.
- The exogenous sources of niacin are meat, fish, eggs, legumes and groundnut. Tryptophan can be converted to niacin in the human body.

- Deficiency of niacin manifests as Pellagra (characterized by diarrhoea, dermatitis and dementia), and is common in alcoholics, HIV patients and tryptophan malabsorption (as is seen in Hartnup disease).

Q. Write briefly about pyridoxine deficiency.

Ans. Pyridoxine maintains sodium and potassium levels, promotes red blood cell production, aids in decreasing the levels of homocysteine and may have a role in preventing cardiovascular problems. Pyridoxine is precursor to pyridoxal phosphate, cofactor for enzyme aromatic amino acid decarboxylase, which is involved in the following reactions:

- 5-Hydroxytryptophan ⟶ Serotonin
- Levodopa ⟶ Dopamine

- **Sources** of pyridoxine include milk, meat, egg yolk, fish, legumes and vegetables.
- The following predispose to pyridoxine deficiency:
  - Pregnancy and infancy (due to increased demand)
  - Alcoholism (acetaldehyde, an alcohol metabolite, induces rapid degeneration of pyridoxine)
  - Drug intake (isoniazid and oestrogen)
- **Manifestations** of pyridoxine deficiency include anaemia, nerve damage, seizures, skin problems and oral ulcers.

Q. Write briefly about the role of diet in carcinogenesis.

Ans. The human diet is a highly complex and variable mixture of naturally occurring and synthetic chemicals. The naturally occurring chemicals include macronutrients (fat, carbohydrate and protein), micronutrients (vitamins and trace metals) and nonnutrient constituents. Several carcinogens and anticarcinogens have been identified in the human diet.

Dietary Carcinogens

These are broadly classified into four categories:

1. **Naturally present carcinogens:** 'Aflatoxin', is an example of a naturally occurring dietary carcinogen. It is a mycotoxin produced by *Aspergillus flavus,* and is implicated in the pathogenesis of hepatocellular carcinoma. Aflatoxicosis is caused by intake of grains and nuts contaminated by the fungus.
2. **Carcinogens forming during food preparation:** Burnt or barbecued foods contain a group of carcinogenic substances called polycyclic aromatic hydrocarbons, which are produced if food is overheated. High intake of fried and broiled food, such as meats, can increase the risk of breast, colon, prostate and pancreatic cancers.
3. **Preservatives and colouring agents added to food:** Artificial sweeteners (like saccharine and cyclamates) are known to cause bladder cancer. Cured, pickled or salty foods contain nitrates, which have been implicated in gastric cancer.
4. **Substances that are converted into carcinogens in the body:** Sodium nitrite which may be present in drinking water and vegetables gets converted to nitrosamine, which is a carcinogen.

Cancer-Preventing Diets

- Fruits and vegetables in the diet are thought to lower the risk of cancer.
- Retinoic acid promotes differentiation of mucous-secreting epithelial cells; therefore, diets containing β-carotene and retinoic acid can reverse metaplastic and precancerous lesions of the respiratory tract.
- High fibre content with low animal fat content in the diet prevents colonic carcinoma (high fat and low fibre content means high level of bile salts and acids in intestine, leading to increased levels of free radicals and carcinogenic byproducts of bile acid metabolism).
- Folic acid, selenium, β-carotene, vitamin C and vitamin E are thought to prevent free radical damage to cell and its DNA; thus, preventing 'cancer initiation'. Vitamin A

enhances immunity and may control free radical production by modulating inflammatory reactions.

Q. Write briefly about obesity.

Ans. Obesity is defined as abnormal or excessive fat accumulation that presents a risk to health. A crude population measure of obesity is the body mass index (BMI)—a person's weight (in kilograms) divided by the square of his or her height (in meters). A person with a BMI of 30 or more is generally considered obese. A person with a BMI equal to or more than 25 is considered overweight. In children, a healthy weight varies with age and sex.

TABLE 9.4. Definitions of overweight (established by World Health Organization and published in 2000)

BMI	Classification
<18.5	Underweight
18.5–24.9	Normal weight
25.0–29.9	Overweight
30.0–34.9	Class I obesity
35.0–39.9	Class II obesity
≥40.0	Class III obesity

The most commonly used definitions, established by the World Health Organization and published in 2000, provide values listed below (Table 9.4). Some modifications to the WHO definitions have been made by particular bodies. Any BMI ≥35 or 40 is *severe obesity.* The neurohumoral mechanisms that regulate the body weight have three components:

1. The afferent system, which generates humoral signals. It is constituted by leptin produced by adipocytes, insulin produced by pancreas and ghrelin produced by the endocrine cells of the stomach.
2. The central processing unit, located primarily in hypothalamus. It integrates afferent signals.
3. The effector system, which carries out 'orders' from hypothalamic nuclei in the form of feeding behaviour and energy expenditure.

Among the afferent signals, insulin and leptin activate catabolic circuits and inhibit anabolic pathways. The levels of ghrelin rise sharply before every meal and fall promptly when the stomach is 'filled'. In fact, it is thought that success of gastric bypass surgery in massively obese individuals may relate more to the associated suppression of ghrelin levels than to an anatomic reduction in stomach capacity. Leptin seems to have a more important role than insulin in the CNS control of calorie balance. Adipocytes communicate with the hypothalamic centres that control appetite by secreting leptin—a member of the cytokine family. When there is an abundance of stored energy in form of adipose tissue, resultant high levels of leptin cross blood–brain barrier, binding to leptin receptors. Leptin receptor signalling has two effects: it inhibits anabolic circuits that normally promote food intake and inhibit energy expenditure. Hence, over a period of time, energy stores (adipocytes) are reduced and weight is lost. This, in turn, reduces circulating levels of leptin, and a new equilibrium is reached. This cycle is reversed when adipose tissue is lost and leptin levels are reduced below a threshold.

Hypoglycaemia induces release of ghrelin, which acts on neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus. The neurotransmitters thus released act on melanin-concentrating hormone (MCH) and orexin to increase appetite and induce adipose tissue deposition.

Overweight and obesity are major risk factors for a number of chronic diseases, including diabetes, metabolic syndrome, cardiovascular diseases and cancer. Once considered a problem only in high-income countries, overweight and obesity are now dramatically on the rise in low- and middle-income countries; particularly, in the urban settings. Obesity

is most commonly caused by a combination of excessive food intake, lack of physical activity and genetic susceptibility, although a few cases are caused due to endocrine disorders, medications or psychiatric illness. Other obesity-associated conditions include gall stones, pancreatitis, abdominal hernia, nonalcoholic steatohepatitis (NASH), gastro-esophageal reflux disease (GERD), polycystic ovarian disease, abnormal pulmonary function, sleep apnoea, deep vein thrombosis, osteoarthritis and gout.

SECTION II

Diseases of Organ Systems

10

Blood Vessels

The blood supply of heart comprises three major vessel types: **arteries** (carry blood from the heart to the systemic circulation); **capillaries** (responsible for exchange of water and chemicals between the blood and tissues); and **veins** (carry blood from capillaries back towards the heart).

Arteries

The **arteries** and **veins** have a similar structure with **three layers, from inside to outside:**

1. ***Tunica intima (thinnest layer)*:** It is composed of a single layer of flattened cells (lining endothelium) held together by a polysaccharide matrix composed of collagen, proteoglycans and elastin. Outside this is present a thin layer of connective tissue (sub-endothelial connective tissue) and circularly arranged elastic bands called internal elastic lamina, which separates the intima from the media.
2. ***Tunica media (thickest layer)*:** This is limited on the inside by the internal elastic lamina and on the outside by another thick elastic band called external elastic lamina (the latter separates the media from adventitia). Tunica media is constituted by connective tissue and polysaccharide substances and is rich in vascular smooth muscle (especially arteries), which controls the calibre of the vessel.
3. ***Tunica adventitia*:** It is made of loose connective tissue and elastic fibres and contains nerves that supply the vessel as well as nutrient capillaries (vasa vasorum). The inner part of the tunica media receives nourishment by direct diffusion of nutrients and oxygen from the lumen whereas the outer part of the media is nourished by the vasa vasorum.

Veins

Veins are different from arteries in the following ways:

1. They have a thinner wall.
2. The three tunicae are less well defined.
3. The elastic tissue is scanty and not well organized into internal and external elastic laminae.
4. The media is richer in collagen and contains less smooth muscle.

Capillaries

These are 7–8 microns in diameter and are lined by endothelial cells which form its tunica intima (inner layer) with pericytes forming its tunica adventitia (outer layer).

Arterioles

They are the smallest branches of the arterial tree which have a diameter less than 100 microns. The intima of an arteriole is composed of endothelial cells which rest on a basement membrane. Larger arterioles may have a fine internal elastic lamina. The arteriolar media is composed of one or two layers of smooth muscle cells and the adventitia is insignificant.

DISEASES OF THE BLOOD VESSELS

Q. Define vasculitis.

Ans. Vasculitis (angiitis) is an inflammatory process involving vessels. Depending on the vessel involved vasculitis is labelled arteritis, capillaritis or venulitis.

Q. Classify vasculitis.

Ans. Vasculitis is classified based on:

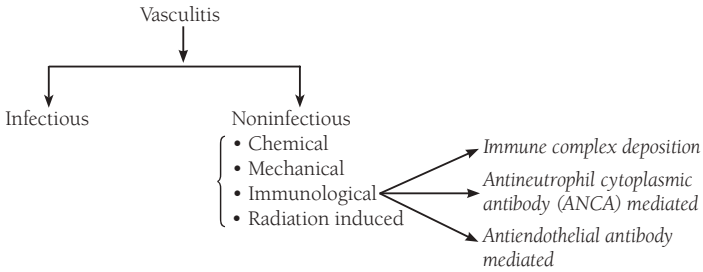
1. **Pathogenesis**
   - (a) Direct infection
     - (i) Bacterial, eg, *Neisseria, streptococci and staphylococci*, *M. tuberculosis* and *M. leprae*
     - (ii) Rickettsial, eg, Rocky Mountain spotted fever
     - (iii) Spirochaetal, eg, syphilis
     - (iv) Fungal, eg, aspergillosis and mucormycosis
     - (v) Viral, eg, herpes zoster and varicella virus
   - (b) Immunologic
     - (i) Immune complex mediated
       - Infection associated, eg, hepatitis B and C viruses
       - Henoch–Schönlein purpura
       - Systemic lupus erythematosus (SLE) and rheumatoid arthritis
       - Drug induced
       - Cryoglobulinaemia
       - Serum sickness
     - (ii) Antineutrophil cytoplasmic antibody (ANCA) mediated
       - Wegener granulomatosis
       - Microscopic polyangiitis (microscopic polyarteritis)
       - Churg–Strauss syndrome
     - (iii) Direct antibody mediated
       - Good pasture syndrome (anti-GBM antibodies)
       - Kawasaki disease (antiendothelial antibodies)
     - (iv) Cell mediated
       - Organ allograft rejection
     - (v) Unknown
       - Giant cell arteritis
       - Polyarteritisnodosa (PAN)
       - Takayasu arteritis
2. **Vessel size** (2012 International Chapel Hill Consensus Conference on the Nomenclature of Vasculitis)
   - (a) Large vessel vasculitis
     - (i) Giant cell (temporal) arteritis: Granulomatous arteritis of the aorta and its major branches, with predilection for extracranial branches of carotid artery
     - (ii) Takayasu arteritis: Granulomatous inflammation of aorta and its major branches
   - (b) Medium-sized vessel vasculitis
     - (i) Polyarteritis nodosa (classic): Necrotizing inflammation of medium-sized or small arteries without glomerulonephritis or vasculitis in arterioles, capillaries or venules
     - (ii) Kawasaki disease: Arteritis involving large-, medium-sized or small arteries, associated with mucocutaneous syndrome, usually seen in children
   - (c) Small vessel vasculitis
     - (i) Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis: Patients with circulating antibodies to neutrophilic cytoplasmic antigens.
     - (ii) Wegener granulomatosis with polyangiitis: Granulomatous inflammation involving the respiratory tract with necrotizing vasculitis affecting small to medium-sized vessels. Necrotizing glomerulonephritis is common.

(iii) Churg–Strauss syndrome (eosinophilic granulomatosis with polyangiitis): Eosinophil-rich granulomatous inflammation involving respiratory tract and necrotizing vasculitis affecting medium- and small-sized blood vessels associated with asthma and blood eosinophilia.
(iv) Microscopic polyangiitis: Necrotizing vasculitis with minimal immune deposits; affects small vessels (necrotizing glomerulonephritis and pulmonary capillaritis are common).

3. **Duration**
   (a) Acute vasculitis
   (b) Chronic vasculitis

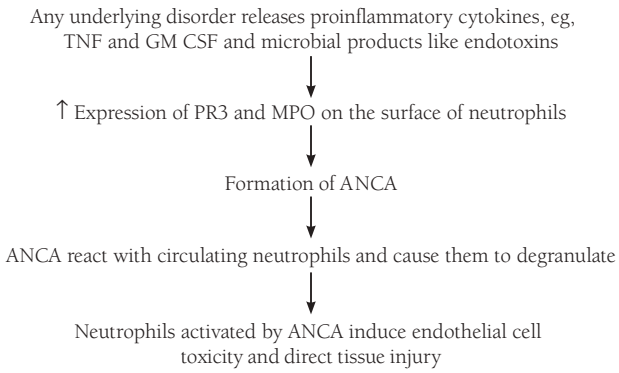
Q. Describe the aetiopathogenesis of vasculitis.

Ans. ***Aetiopathogenesis of vasculitis*** *(Flowchart 10.1)*



FLOWCHART 10.1. Aetiopathogenesis of vasculitis.

1. **Infectious vasculitis**
   (a) Direct invasion of the artery by the infectious agents, especially bacteria and fungus
   (b) May be found in the vicinity of an infected focus like tuberculosis and pneumonia
   (c) May arise from haematogenous spread of infection, as in infective endocarditis or septicaemia
2. **Noninfectious vasculitis (chemical, mechanical, immunological and radiation induced):** Majority, immune mediated. Main immunological mechanisms that initiate noninfectious vasculitis are
   (a) **Immune complex deposition:** May be of two types:
      (i) Local immune complex formation: The antigen diffuses into the vessel wall and the antibody is brought from the circulating blood. Antigen and antibody react in the vessel wall to form immune complexes, which activate the complement system (seen in polyarteritisnodosa and simulates Arthus reaction).
      (ii) Deposition of circulating immune complexes in the vessel wall: Immune complexes circulating in the blood may get deposited in the wall of small blood vessels to activate complement and inflammatory cells (seen in SLE).
   (b) **ANCA:**
      (i) ANCA are autoantibodies directed against the enzymes mainly found within the azurophilic (primary) granules in neutrophils and lysosomes of monocytes and endothelial cells (Flowchart 10.2).
      (ii) Levels of ANCA reflect the degree of disease activity.
      (iii) Classified into two types based on immunofluorescence patterns:
         - Anti-proteinase 3 (PR3–ANCA): Previously called **c-ANCA.** The target antigen is proteinase 3 **(PR3)**, a neutrophil granule constituent which share antigenic structure with some microbial peptides. It is therefore hypothesized that PR3-ANCA is generated following some fungal infections.
         - Antimyeloperoxidase (MPO–ANCA): Previously called **p–ANCA,** Myeloperoxidase (**MPO**); seen in microscopic polyangiitis and Churg–Strauss syndrome, is thought to be induced by some therapeutic agents.
   (c) **Antiendothelial cell antibodies:** Induced by defects in immune regulation; seen in SLE and Kawasaki disease. Immunologic mechanisms responsible for most cases are:
      (i) Type III hypersensitivity
      (ii) Type IV hypersensitivity (as in granulomatous inflammation)



FLOWCHART 10.2. Mechanism of ANCA induced vasculitis.

Q. Write briefly about Takayasu arteritis.

Ans. Takayasu arteritis is a granulomatous vasculitis which typically involves the medium and large sized arteries.

Salient Features:

- Also called '**pulseless disease**', it chiefly affects the aorta and its major branches (**aortic-arch syndrome**).
- The orifices of the major arteries to the upper portion of the body are markedly narrowed or obliterated.

Pathogenesis

Autoimmune reaction to aortic tissue.

Clinical Features

- Its clinicomorphology overlaps with giant cell arteritis; however, unlike the latter, which is usually seen in women over 50 years, Takayasu arteritist affects younger women.
- Manifests initially with nonspecific symptoms (fever, weight loss and fatigue); may present later with marked lowering of blood pressure and weaker pulses in upper extremities along with ocular disturbances and neurological deficit. More distal involvement leads to claudication of the legs.

Gross Pathology

Aortic wall is irregularly thickened, and intima is wrinkled.

Microscopy

Shows severe transmural granulomatous inflammation with giant cells and patchy necrosis in the tunica media.

Q. Write briefly about temporal (giant cell) arteritis.

Ans. Temporal arteritis is an inflammatory disease affecting arteries of the head.

Salient Features

- Typically causes granulomatous inflammation of medium- and large-sized arteries.
- Cranial (temporal, ophthalmic and common carotid), axillary, brachial and femoral arteries are commonly involved.
- Affects adults more than 70 years of age, with a slight female preponderance.

Pathogenesis

Attributed to T-cell-mediated immunologic reaction against arterial wall components.

Clinical Features

Symptoms vary from being nonspecific (fever, fatigue and weight loss) to severe headache and blindness (caused due to ophthalmic artery involvement).

Gross Pathology

The affected arteries are thickened and cord like, with narrowing of the lumina.

Microscopy

- Sections show chronic granulomatous reaction with giant cells, usually around the internal elastic lamina, typically involving the entire circumference of the vessel wall.
- Internal elastic lamina is fragmented with presence of giant cells of foreign body or Langhans type.
- Eccentric or concentric intimal cellular proliferation may cause luminal narrowing.

Q. Write briefly about polyarteritis nodosa (PAN).

Ans. Polyarteritis nodosa is a systemic necrotizing vasculitis involving small- and medium-sized muscular arteries.

Salient Features of Polyarteritis Nodosa

- Affects adults (males more commonly affected than females).
- Kidneys, heart, liver, GIT, muscle, pancreas, testes, nervous system and skin are usually involved with sparing involvement of pulmonary or glomerular vessels.
- Hypertension

Pathogenesis

Deposition of immune complexes and hepatitis B antigenaemia is implicated.

Clinical Features

(due to ischaemia and infarction of the affected tissues and organs)

- Fever, malaise, weakness and weight loss
- Renal manifestations (albuminuria and haematuria)
- Abdominal pain and melena
- Peripheral neuritis

Gross Pathology

- Transmural (involvement of whole thickness of the vessel wall) and segmental (involvement of only a portion of the vessel circumference) vasculitis of small- and medium-sized muscular arteries.
- Predilection for bifurcations and branching points.
- Segmental erosion with weakening of arterial wall may cause aneurysmal dilation or localized rupture.

Microscopy

- ***Acute stage*:** Transmural inflammation (chiefly neutrophils and eosinophils) with fibrinoid necrosis
- ***Healing stage*:** Fibroblastic proliferation with chronic inflammation (lymphocytes, plasma cells and macrophages)
- ***Healed stage*:** Thickened arterial wall due to dense fibrosis. Haemosiderin-laden macrophages and organized thrombi may be seen

All stages of inflammation can be present at the same time.

Q. Write briefly about Kawasaki disease.

Ans. Kawasaki disease was originally reported in Japan and is the foremost cause of heart disease affecting children.

Salient Features

- Self-limiting acute febrile illness of childhood (majority patients are less than 4 years)
- Affects large- to medium-sized to small arteries

Pathogenesis

Unknown; infectious agents are thought to trigger the disease in genetically predisposed individuals

Clinical Features

- Conjunctival and oral erythema, erythema of palms and soles
- Oedema of hands and feet
- Desquamative rash and cervical lymph node enlargement (mucocutaneous syndrome)
- Commonly involves the coronary arteries to form aneurysms which may rupture resulting in acute myocardial infarction

Pathology

- Transmural inflammation like PAN; however, less fibrinoid necrosis
- Most changes in cardiovascular system. Segmental erosion with weakening of arterial wall may cause aneurysmal dilation, thrombosis or localized rupture.

Q. Write briefly about microscopic polyangiitis.

Ans. Microscopic polyangiitis is a necrotizing vasculitis also known as hypersensitivity or leukocytoclastic vasculitis.

Salient Features

- Characterized by inflammatory involvement of venules, capillaries and arterioles
- Skin, mucous membrane, lungs, brain, heart, GIT, kidneys and muscles are commonly affected
- Necrotizing glomerulonephritis and pulmonary capillaritis are particularly common (feature differentiating it from PAN; also, microscopic polyangiitis affects smaller vessels and all its lesions are in the same histopathological stage, unlike PAN)
- Typically, presents as palpable purpura, haemoptysis, arthralgias, abdominal pain, haematuria, proteinuria, muscle pain or weakness

Pathogenesis

Thought to be due to an immunologic response to an antigen that may be bacteria (streptococci), viruses, malarial parasite, drugs (penicillin) and chemicals. Antibodies are formed leading to immune complex formation or development of secondary ANCA-associated immune responses.

Clinical Features

Depending on the vessel involved, patient may present with:

- Haemoptysis, albuminuria and haematuria
- Abdominal pain and melena
- Muscle pain and weakness
- Palpable purpura

Microscopy

Shows segmental and focally transmural lesions which involve smallest vessels, sparing medium-sized and large arteries. Two histological forms are seen:

- ***Leukocytoclastic vasculitis*:** Vasculitis is due to immune complex deposition. There is fibrinoid necrosis with neutrophilic infiltrate in the vessel wall. Many neutrophils appear fragmented.
- **Lymphocytic vasculitis:** Vascular injury occurs due to lymphocyte-macrophage-mediated delayed hypersensitivity.

Q. Write briefly about Churg–Strauss syndrome (also called 'allergic granulomatosis and angiitis').

Ans. Churg–Strauss syndrome is a rare small vessel vasculitis.

Salient Features

- It is a multisystem disease characterized by necrotizing vasculitis with granulomas and eosinophilic necrosis.
- Has a strong association with allergic rhinitis, bronchial asthma, lung infiltrates and eosinophilia.
- Commonly affects vessels in the lungs, heart, spleen, peripheral nerves and skin. Renal disease is less frequent.
- Coronary arteritis and myocarditis are the main causes of death.

Pathogenesis

Thought to result from hyperresponsiveness to an allergic stimulus. ANCAs are positive in 50% cases.

Clinical Features

- Palpable purpura (due to involvement of skin)
- Gastrointestinal bleeding
- Focal and segmental glomerulosclerosis
- Cardiomyopathy (in >50% patients)

Microscopy

- Infiltration of vessels and perivascular tissue by eosinophils without overt vasculitis in the early phase.
- Intravascular and extravascular granulomas with vasculitis in later stage.

Q. Write briefly about Wegener granulomatosis (granulomatosis with polyangiitis).

Ans. Wegener granulomatosis is a necrotizing vasculitis characterized by a clinicopathological triad of:

1. Necrotizing granulomas of the upper respiratory tract (ear, nose, sinuses and throat) and/or lower respiratory tracts (lungs).
2. Focal necrotizing granulomatous vasculitis of the small- to medium-sized vessels.
3. Focal necrotizing crescentic glomerulonephritis.

Pathogenesis

- May represent a T-cell-mediated hypersensitive response to some inhaled infectious or environmental agent.
- Characterized by presence of immune complexes (in vessel wall and glomeruli) and PR3–ANCA, which is a good marker of the disease.

Clinical Features

- Usually affects adult males, in the 5th decade; multiple organ involvement may be seen.
- Clinical manifestations include:
  - Persistent pneumonitis with bilateral infiltrates in the lungs
  - Chronic sinusitis and nasopharyngeal ulceration
  - Chronic renal disease, skin rashes, muscle pain and articular involvement
- Wegener granulomatosis without renal involvement is labelled 'limited form' of the disease.
- Untreated, 80% of the patients die within a year.

Microscopy

- Necrotizing vasculitis (segmental or circumferential) of small and sometimes large vessels
- Granulomatous inflammation with geographic pattern of necrosis with extensive infiltration by neutrophils, mononuclear cells, epithelioid cells, multinucleate giant cells and fibroblasts
- Dispersed granulomas may coalesce to form nodules that undergo cavitation
- Renal lesions may be focal or diffuse, namely, focal necrotizing (acute focal proliferation and necrosis in the glomeruli with thrombosis) and diffuse crescentic glomerulonephritis

Q. Write briefly about Raynaud phenomenon.

Ans. Raynaud phenomenon is not a true vasculitis, but a functional vasospastic disorder chiefly affecting small arteries and arterioles of the extremities of young healthy females.

- Clinically, it presents with pallor, redness and cyanosis of the digits and tips of nose or ears.
- Cause is not known; it is thought to be due to vasoconstriction mediated by autonomic stimulation of the affected vessels.
- Ischaemic effect is provoked by cold (emotions, trauma, hormones and drugs also play a role).
- No pathological change is observed in vessel wall except mild intimal thickening later in the course of the disease.
- Raynaud phenomenon can be primary or secondary. The latter occurs due to an underlying cause, eg, atherosclerosis, connective tissue disease, multiple myeloma and Buerger disease. Primary Raynaud phenomenon differs from secondary Raynaud phenomenon in having symmetrical involvement of the extremities.

Q. Write briefly about Buerger disease.

Ans. Also called thromboangiitis obliterans; it is characterized by acute and chronic, segmental, thrombosing, inflammatory involvement of the small- and medium-sized arteries and veins of extremities.

- Usually affects men younger than 35 years, particularly the heavy smokers.
- Intermittent claudication due to ischaemia manifests as intense pain in the limbs.
- Fibrous tissue cuffs may form around arteries, veins and nerves leading to gangrene.

Aetiopathogenesis

- Heavy cigarette smoking directly damages endothelium leading to hypercoagulability and thrombosis.
- An immune response to some constituents of tobacco smoke has also been implicated.
- Familial occurrence, ethnic distribution and HLA association point to a possible genetic basis.

Microscopy

- **Early stage:** Polymorphs in all the layers of vessels accompanied by thrombosis in the lumen
- **Advanced stage:** Mononuclear infiltrate with fibrosis

Q. Describe the salient features of phlebothrombosis (thrombophlebitis).

Ans. Salient features of phlebothrombosis

- Deep leg veins are involved in more than 90% cases followed by periprostatic venous plexus in males and pelvic venous plexus in females.
- Cardiac failure, genetic hypercoagulability syndromes, neoplasia, pregnancy, obesity, post-operative state, prolonged bed rest and immobilization are the most important clinical predispositions.
- Migratory thrombophlebitis (**Trousseau sign**) is seen in adenocarcinomas of pancreas, colon and lung. Hypercoagulability may occur as part of a paraneoplastic syndrome. The resultant venous thrombosis has a tendency to appear in one site only to disappear, followed by thromboses in other veins (migration).
- Thrombi in the legs present with:
  - Local manifestations
    - Oedema distal to an occluded vein
    - Cyanosis
    - Dilatation of superficial veins
    - Heat, tenderness, redness, swelling and pain

    Note: Above features may be absent in bedridden patients. In these patients, pain is elicited by applying pressure over affected veins, squeezing the calf muscles, or forced dorsiflexion of foot (**Homans sign**).
  - Pulmonary embolism
    - Common and serious manifestation (not infrequently may be the first manifestation). It is due to the contraction of the surrounding vessels, which tends to displace the thrombi from their attachment.

Q. Write briefly about superior vena caval syndrome.

Ans. It is usually caused by a neoplasm that compresses and invades superior vena cava, most commonly bronchogenic carcinoma and mediastinal lymphoma. Manifests as dusky cyanosis, dilatation of veins of head, neck and arms. Pulmonary vessels may also get compressed causing respiratory distress.

Q. Write briefly about inferior vena caval syndrome.

Ans. Many neoplasms, eg, hepatocellular and renal cell carcinoma, show a striking tendency to grow within veins with ultimate extension into IVC (occasionally into right atrium). It manifests with marked oedema of legs, distension of superficial collateral veins of lower abdomen and massive proteinuria (due to renal vein involvement).

Q. Write briefly about lymphangitis and lymphoedema.

Ans. Primary lymphangitis is an extremely uncommon primary disorder of the lymphatic vessels. Secondary lymphangitis and lymphoedema develop in association with inflammation and cancer. **Lymphangitis** may be acute or chronic depending on its duration.

- **Acute lymphangitis** is caused by bacterial infections, most commonly β-haemolytic streptococci (Flowchart 10.3).
- **Chronic lymphangitis:** Results from persistence of acute lymphangitis or chronic infections, eg, tuberculosis, syphilis and actinomycosis.

β-haemolytic streptococcal infection

↓

Lymphatics become dilated with exudate (neutrophils and histiocytes), which extends through the wall into perilymphatic tissue

↓

Cellulitis or focal abscess formation (clinically, manifests as painful subcutaneous red streaks that extend along the course of lymphatics with painful enlargement of lymph nodes)

FLOWCHART 10.3. Pathogenesis of acute lymphangitis.

- **Primary (idiopathic) lymphoedema** may occur as an **isolated congenital defect** (simple congenital lymphoedema), as **Milroy disease** (heredofamilial congenital lymphoedema) or as **lymphoedema praecox** (affects young females; presents with oedema of unknown cause, which usually begins in the feet and slowly accumulates to cause swelling of the involved extremity to many times normal size with superimposed infections and chronic ulceration).
- **Obstructive (secondary) lymphoedema:** Occurs secondary to occlusion of lymphatic drainage due to spread of malignant tumours, radical surgical procedures, eg, radical mastectomy with axillary dissection, postirradiation fibrosis, filariasis and postinflammatory thrombosis, and scarring.
- **Chylous ascites, chylothorax and chylopericardium:** Caused by rupture of dilated lymphatics into the respective cavities.
- Persistence of lymphoedema leads to subcutaneous interstitial fibrosis with enlargement of the affected part and induration called '**peau d'orange**' appearance.

Q. Define aortic dissection. Describe in brief its pathology and clinical consequences.

Ans. Aortic dissection (dissecting haematoma) is a catastrophic illness characterized by the forceful separation of the planes of the media with the formation of an intramural hematoma within the vessel wall, which may rupture outside causing massive haemorrhage.

Salient Features

- Unlike an aneurysm, aortic dissection may or may not be associated with dilatation of the vessel (therefore, the older term '**dissecting aneurysm**' is discouraged).
- Most common site is ascending **aorta**.
- Affects two age groups:
  - Hypertensive men between 50 and 70 years
  - Younger patients with a connective tissue abnormality, which affects the aorta, eg, Marfan syndrome (genetic defect in fibrillin, a connective tissue protein responsible for elastic tissue formation)
- Aortic dissection can be iatrogenic in origin (trauma during cardiac catheterization or bypass surgery).
- Rarely, dissection of aorta occurs during or following pregnancy (due to haemodynamic stress of pregnancy induced vascular remodelling).

Types

- Aortic dissections are generally classified into two types:
  - Type A: More common and more serious proximal lesions involving ascending aorta only or ascending and descending aorta (types I and II of DeBakey classification; Figure 10.1A).
  - Type B: Distal lesions not involving the ascending part and beginning distal to the subclavian (type III of DeBakey classification; Figure 10.1B).

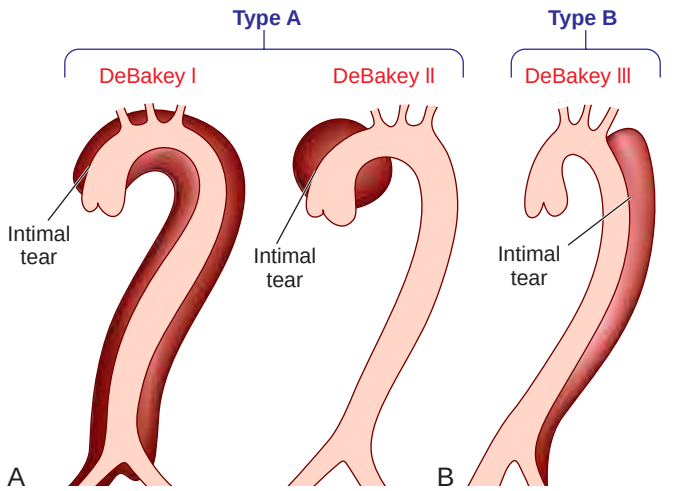


FIGURE 10.1A and B. Types of aortic dissection.

Clinical Effects

- Aortic dissection presents with sudden severe pain, beginning in anterior chest and radiating to the back.
- May rupture leading to haemorrhage into body cavities (pleural, pericardial or peritoneal).
- Retrograde dissection into the aortic root can cause disruption of the aortic valve.
- Cardiac tamponade, aortic insufficiency and myocardial infarction may be seen.
- Compression of spinal arteries may cause transverse myelitis.

Pathologic Changes

- The most common histopathological lesion is **cystic medial degeneration.**
- Dissection is seen between the outer and middle third of aortic media; so that blood separates the intima and inner two-thirds of the media on one side from the outer one-third of the media and the adventitia on the other.
- In 10% of dissecting aneurysms, a second intimal tear is seen in the distal part of the dissection, so that the blood enters the false lumen through the proximal tear and reenters the true lumen through the distal tear **(double barrel aorta).**
- If the patient survives, the false lumen may develop endothelial lining **(chronic dissection).**

Q. Define and classify aneurysms. Summarize their morphology and clinical consequences.

Ans. An aneurysm is a permanent abnormal dilatation of a blood vessel occurring due to congenital or acquired weakening or destruction of the vessel wall. It is commonly seen in large elastic arteries especially aorta and its major branches.

Classification

1. **Depending on the composition of wall** (Fig. 10.2)
   (a) True aneurysm: Composed of all the layers of vessel wall or thinned out wall of the heart
   (b) False or pseudoaneurysm: A breach in the vascular wall leads to formation of an intravascular haematoma, which has a fibrous wall, and occurs secondary to trauma

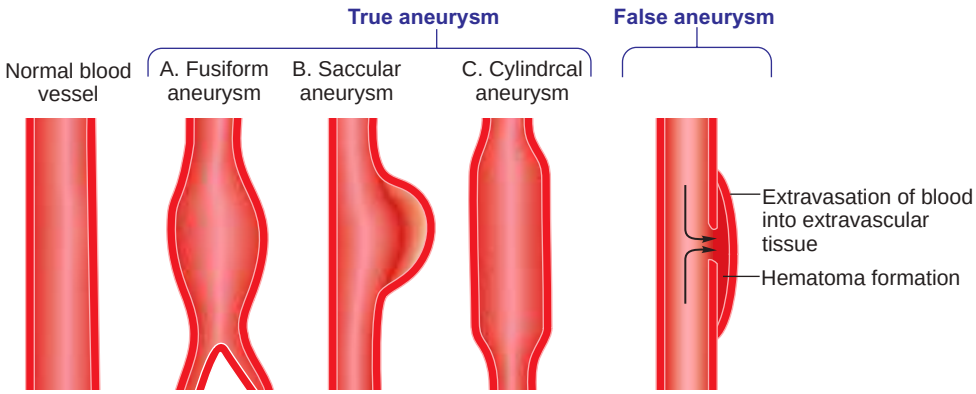


FIGURE 10.2. Types of aneurysms.

2. **Depending on the shape** (Fig. 10.2)
   (a) Saccular: Large spherical outpouching
   (b) Fusiform: Spindle-shaped dilatation
   (c) Cylindrical: Continuous parallel dilatation
   (d) Serpentine or varicose: Tortuous dilatation
   (e) Racemose: Mass of intercommunicating small arteries and veins
3. **Based on pathogenetic mechanisms**
   (a) Atherosclerotic (arteriosclerotic) aneurysm (most common type)
   (b) Syphilitic (luetic) aneurysm (found in the tertiary stage of syphilis)
   (c) Dissecting aneurysm (dissecting haematoma) in which blood enters the wall of the vessel
   (d) Mycotic aneurysm (weakening of the arterial wall by microbial infection)
   (e) Berry aneurysm (malformation located in Circle of Willis in the base of the brain)

Aneurysms most commonly encountered in clinical practice are as follows:

1. **Abdominal aortic aneurysm (AAA)**
   - Most common form of aortic aneurysms, usually seen in males more than 50 years.
   - Most common location is abdominal aorta (other locations are thoracic aorta [ascending and arch of aorta], iliac artery and large systemic arteries).
   - Most common cause is atherosclerosis.

Pathogenesis

- **Severe atherosclerosis** (thinning and destruction of the medial elastic tissue causes atrophy and weakening of the vessel wall eventually leading to aneurysm formation)
- **Genetic predisposition** (genetic defects lead to inadequate or abnormal synthesis of connective tissue component as in Marfan and Ehlers–Danlos syndrome)
- **Abnormality in matrix metalloproteinases** (decreased level of tissue inhibitors of metalloproteinases, ie, TIMP) which disturbs the balance between collagen synthesis and degradation
- **Infections** (bacterial, mycotic leading to suppuration or syphilitic leading to endarteritis obliterans and ischaemia)

Pathologic changes

- Variable sized, usually larger than 5–6 cm
- Most frequently fusiform or saccular
- Lumen may contain a mural thrombus
- Two variants of abdominal aortic aneurysms:
  - Inflammatory: Less frequent; characterized by dense periaortic fibrosis containing lymphocytes, plasma cells and macrophages; thought to be due to a localized immune response to wall of abdominal aorta
  - Mycotic: Infected by circulating organisms which cause suppuration

Clinical consequences

- Rupture into peritoneal cavity causing fatal haemorrhage
- Vascular obstruction of a branch of aorta, eg, renal, mesenteric, vertebral leading to ischaemic injury in the kidney, GIT and spine, respectively
- Embolism from atheroma or mural thrombus
- Compression of ureter or erosion of a vertebra presenting as pain, which is deep, boring, visceral and felt most prominently in the lumbosacral region
- Presentation as a palpable pulsatile abdominal mass

2. **Thoracic aortic aneurysms**

Pathogenesis

- Most commonly associated with hypertension
- May also be associated with connective tissue disorders, eg, Marfan syndrome

Pathologic changes

Weakening of the vessel wall leading to progressive dilatation

Clinical consequences

- Breathing difficulty (due to compression of lung and airways)
- Difficulty in swallowing due to compression of oesophagus
- Chronic cough (due to compression of recurrent laryngeal nerve)
- Costovertebral pain (due to erosion of a rib or vertebra)
- Aortic valvular dilation and insufficiency leading to heart failure
- Catastrophic blood loss due to rupture

Q. Define arteriosclerosis.

Ans. The term 'arteriosclerosis' is synonymous with 'hardening of the arteries' which indicates reduced elasticity and thickening of arterial walls. Arteriosclerosis has three main histopathological patterns:

1. **Atherosclerosis** is the most common and clinically significant pattern of arteriosclerosis in which there is formation of intimal fibrofatty plaques.
2. **Monckeberg medial calcific sclerosis** is an entity in which there are calcific deposits in muscular arteries of the elderly. These deposits are often visible on imaging but do not narrow the vessel lumen and therefore have little clinical significance.
3. **Hyaline** and **hyperplastic arteriolosclerosis** affect small arteries and arterioles and are mostly seen associated with hypertension and diabetes mellitus.

Q. Define atherosclerosis. Enumerate the risk factors involved in the pathogenesis of atherosclerosis.

Ans. Atherosclerosis is characterized by formation of the **fibrofatty plaques** affecting primarily the intima of large- and medium-sized muscular arteries (aorta, coronary and cerebral).

Risk Factors

1. **Major risk factors**
   (a) Constitutional (nonmodifiable)
      (i) Age: Early lesions of atherosclerosis may be present in childhood, but clinically significant lesions are found with increasing age.
      (ii) Sex: Males are more commonly affected than females; atherosclerosis is uncommon in premenopausal women. Increased incidence in postmenopausal women was thought to be due to falling oestrogen levels; however, oestrogen replacement therapy in older women has not been found to decrease the cardiovascular risk.
      (iii) Genetic factors: Hereditary genetic derangements of lipoprotein metabolism, which predispose the individual to high blood lipid level like familial hypercholesterolaemia have been implicated.
      (iv) Familial and racial factors: The established predisposition to ischaemic heart disease is multifactorial in origin and is related to the presence of other risk factors like diabetes and hypertension which show familial clustering. Blacks have less severe atherosclerosis than whites.
   (b) Acquired (potentially modifiable)
      (i) Hyperlipidaemia:
         - Major classes of lipoproteins are chylomicrons, VLDL (very low density lipoproteins), low density lipoproteins (LDL) and high density lipoproteins (HDL).
         - LDL delivers cholesterol to peripheral tissues (bad cholesterol) and HDL removes cholesterol from the tissues to deliver it to the liver to finally be excreted in the bile (good cholesterol).
         - Diets containing large quantities of saturated fats raise the plasma cholesterol levels. Also, transfats which form due to artificial hydrogenation of polyunsaturated oils (as in baking) are immensely harmful.
         - Diets rich in polyunsaturated fats and omega-3-fatty acids lower the plasma cholesterol levels.
         - Most evidence implicates hypercholesterolaemia:
            - Atherosclerotic plaques contain cholesterol and cholesterol esters.
            - Individuals with hypercholesterolaemia, eg, patients of diabetes mellitus, myxoedema and nephrotic syndrome, have increased risk of developing atherosclerosis.
            - Dietary regulation and cholesterol-reducing drugs have beneficial effects.

         Note: Main lipids in blood are cholesterol (normal 140–200 mg/dL; borderline, 240 mg/dL) and triglycerides (normal <160 mg/dL). Elevation of serum cholesterol >260 mg/dL in men and women causes three times higher risk of heart disease.
      (ii) Hypertension: Major risk factor at all ages
      (iii) Diabetes mellitus: Atherosclerosis manifests faster in both Types I and II diabetes mellitus
      (iv) Smoking: Men who smoke a pack of cigarettes a day are 3–5 times more likely to die of IHD (ischaemic heart disease) than nonsmokers
2. **Minor risk factors**
   (a) **Inflammation** is an integral part of evolution of atherosclerosis and is very closely linked to its development. C-reactive protein (CRP), a marker of inflammation, has been found to be one of the most sensitive predictors of ischaemic heart disease.
   (b) **Obesity:** Abdominal/central obesity has been found to be an important risk factor.
   (c) **Metabolic syndrome:** Characterized by insulin resistance, glucose intolerance, hypertension, central obesity, dyslipidaemias, endothelial dysfunction, increased oxidative stress and a systemic inflammatory state, which predisposes to thrombosis.
   (d) **Lipoprotein (a) levels:** Lipoprotein (a) is an aberrant form of LDL that has the apolipoprotein B-100 portion of LDL linked to apolipoprotein A. Increased levels predispose to cardiovascular events.
   (e) **Factors affecting haemostasis:** Several factors associated with coagulation and fibrinolysis are important predictors of cardiovascular events, eg, increased levels of plasminogen activator inhibitor is associated with myocardial infarction and stroke.
   (f) **Physical inactivity and lack of exercise:** A sedentary lifestyle predisposes to atherosclerosis.

(g) **Lifestyle:** Type A behaviour characterized by the aggressiveness, competitiveness and drive have increased risk.
(h) **Hyperhomocystinaemia and homocystinuria:** High levels of circulating homocysteine may lead to endothelial injury and vascular disease.

Q. Write in brief on the pathogenesis of atherosclerosis.

Ans. The exact pathogenesis of atherosclerosis is not known; it is thought to be a multifactorial disease. Many theories have been put forward:

- **Encrustation hypothesis proposed by Rokitansky in year 1852:** Atheroma represents lipid encrustation on the arterial wall and formation of thrombus by components of blood (platelets, fibrin and leukocytes).
- **Insudation hypothesis proposed by Virchow in year 1856:** There is cellular proliferation of intimal cells due to increased imbibing of lipids from blood ('lipid theory').
- **Currently, the most accepted theory in circulation is reaction/response-to-injury hypothesis (**Flowchart 10.4**).** This theory identifies atherosclerosis as a chronic inflammatory and healing response to endothelial injury.

Endothelial cell injury
(endothelial dysfunction or denudation due to hyperlipidaemia, hypertension, toxins in cigarette smoke, viruses and other infective agents, haemodynamic disturbances, immune complex deposition, irradiation, homocysteine, etc.)
↓
Platelet aggregation and platelet release reaction
↓ ↑ *VCAM-1, IL-1 and TNF*
Circulating monocytes and lymphocytes (both CD4+ and CD8+ T cells) adhere to the area of injury and emigrate into the vessel wall (monocytes become macrophages)
↓
The above cells release various cytokines (growth factors) some of which induce smooth muscle proliferation and direct chemotaxis of the smooth muscle cells to the intimal area of the vessel
↓
Macrophages, lymphocytes and smooth muscle cells imbibe LDL-containing cholesterol and become **foam cells** with subsequent development of fatty streaks (reversible lesions)
↓
Injured endothelial cells and macrophages also produce free radicals, which induce formation of oxidized LDL, a potent enhancer of the atherosclerotic process (oxidized LDL acts to attract, proliferate, immobilize and activate monocytes and is cytotoxic for endothelium and smooth muscle cells; it is ingested by macrophages through scavenger receptors different from LDL receptors)
↓
Fatty streaks continue to enlarge and eventually disrupt the endothelial surface
↓
Platelets adhere to the damaged endothelium overlying the fatty streaks and increase PDGF, which further contributes to smooth muscle proliferation
↓
Over time, the proliferating smooth muscle cells located at the base of the fatty streak begin to synthesize collagen, elastin and proteoglycans, which subsequently produce fibrous plaques
↓
Fibrous plaques undergo dystrophic calcification, haemorrhage, thrombosis, fissuring and ulceration to form a complicated atheromatous plaque

FLOWCHART 10.4. Reaction/response-to-injury hypothesis.

Q. Describe the pathological features and clinical consequences of atherosclerosis.

Ans. Early lesions show diffuse intimal thickening. Fatty streaks are the forerunners in the evolution of atherosclerotic plaques.

1. **Fatty streaks and dots**
   Salient features:
   - Start by themselves and are harmless, but are considered earliest precursors of atheromas.
   - Usually begin in the first year of life, and are present in all children older than 10 years.
   - Especially prominent in the aorta and other major arteries and are associated with the known risk factors of atherosclerosis.

   ***Gross*:** Multiple flat or slightly elevated, yellow intimal spots less than 1 mm in diameter, which coalesce into elongated streaks, 1 cm or longer.

   ***Microscopy*:** Composed of lipid-laden macrophages (foam cells) and a few T lymphocytes.
2. **Atheromatous plaques** (Fig. 10.3)**:** Fully developed lesions, also called fibrous plaque, fibrofatty plaque or atheroma

   ***Gross*:** White to yellowish-white, 1–2 cm lesion raised above the luminal surface. Has a grey-white fibrous cap and a central core of yellowish white soft, grumous lipid.

   ***Microscopy*:** Depends on age of the lesion
   - **Superficial (luminal) part of fibrous cap** is composed of smooth muscle cells and collagen.
   - **Cellular area under and to the side of the fibrous cap (shoulder of the lesion)** is more cellular and composed of foamy macrophages, T lymphocytes and few smooth muscle cells.
   - **Deeper (central) soft core** is located deep to the cap and is composed of extracellular lipid material, cholesterol clefts (needle-shaped, cleft-like spaces), fibrin, necrotic debris and lipid-laden foam cells.
   - **Older advanced lesions** show dense hyalinized collagen, fibrous tissue and smooth muscle cells.

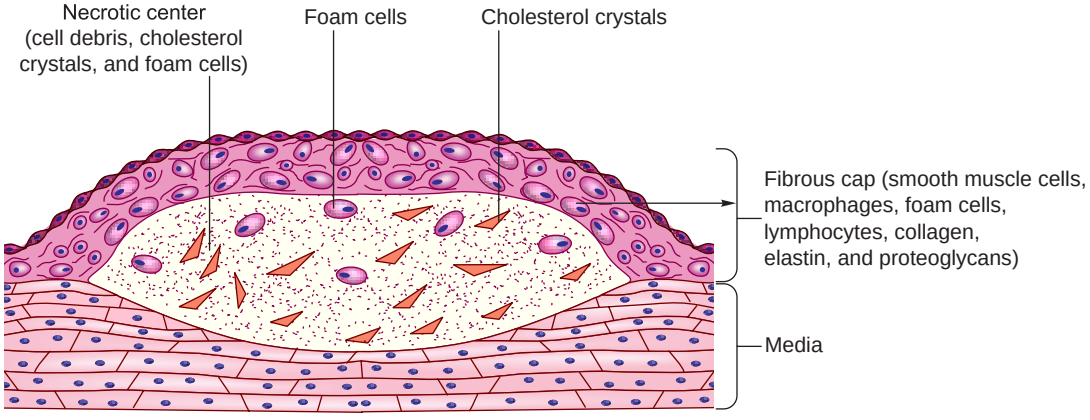


FIGURE.10.3. Diagrammatic representation of an atheroma.

Clinical Features of Atherosclerosis

- Most often and most severely affected are elastic arteries like abdominal aorta, carotids and iliac; and large- and medium-sized muscular arteries like coronary and popliteal.
- Symptomatic plaques most often involve arteries supplying the heart, brain, kidneys and lower extremities.
- Major clinical consequences of atherosclerosis are:
  - Myocardial infarction (heart attack)
  - Cerebral infarction (stroke)
  - Intermittent claudication and peripheral vascular disease (gangrene) of lower extremities
  - Ischaemic bowel disease, infarction and ischaemic strictures of intestine
  - Renovascular hypertension

Complications of Atherosclerosis

1. **Calcification:** Occurs in advanced plaques, especially in aorta and coronaries. The diseased intima crackles like egg shell when incised.
2. **Ulceration:** Layers covering the soft pultaceous material of an atheroma may ulcerate due to trauma or haemodynamic force.
3. **Thromboembolization:** Thrombosis occurs due to ulceration of the plaque and endothelial damage. Emboli composed of lipid material and debris may arise from the thrombi.
4. **Haemorrhage:** Originates either from the luminal blood or rupture of thin capillaries in adventitia.
5. **Aneurysm formation:** Severe atherosclerosis causes atrophy and thinning of media with fragmentation of internal elastic lamina resulting in weakening of vessel wall and aneurysm formation.
6. **Progressive plaque growth:** Causes critical stenosis and obstruction of the vessel.

Q. Differentiate between fatty streak and atheroma.

Ans. Differences between fatty streak and atheroma are summarized in Table 10.1.

TABLE 10.1. Differences between fatty streak and atheroma

Features	Fatty streak	Atheroma
Age affected	Starts in children as young as 1 year	Affects older individuals
Composition	Lipid accumulation is mainly intracellular (lipid filled foam cells); T lymphocytes and extracellular lipid is present in small amounts	Large core of extracellular lipid
Gross appearance	• Multiple yellow, flat lesions less than 1 mm in diameter	• Whitish yellow, raised, usually eccentric lesions, measuring 0.5–1.5 cm in diameter
	• Do not encroach upon the lumen	• Encroach upon the lumen
Vasculature involved	May be distributed in areas other than those generally affected by atherosclerosis	Primarily affects elastic as well as large- and medium-sized muscular arteries
Geographic distribution	May be seen in geographic areas, which have a low incidence of atherosclerosis	Common in Western world and developed countries
Clinical consequences	Does not generally cause any obstruction to blood flow	May cause myocardial and cerebral infarction, aortic aneurysms and peripheral vascular disease

Q. Classify hypertension. Write briefly about the factors regulating blood pressure and describe its morphological effects.

Ans. The clinical classification of hypertension is given in Table 10.2.

TABLE 10.2. Clinical classification of hypertension

Category	Systolic (mm Hg)	Diastolic (mm Hg)
Normal	90–119	60–79
Prehypertension	120–139	80–89
Hypertension		
1. Stage I	140–159	90–99
2. Stage II	>160	>100
Malignant hypertension	>200	>140
Isolated systolic hypertension	>140	< 90

- Blood pressure varies with many factors such as age, exercise, emotional disturbances like fear and anxiety, so should be measured twice during two separate examinations under least stressful conditions.
- Industrialized countries have a higher prevalence of hypertension.
- Hypertension is more common amongst males and Afro-Americans.
- The control of hypertension reduces mortality due to stroke and coronary artery disease.
- Systolic blood pressure correlates with stroke volume and the compliance of the aorta.
- Factors determining **systolic pressure** include **preload** (volume of blood in the left ventricle), **afterload** (resistance against ejection of blood from the left ventricle) and **contractility of the heart**. The ability of the aorta to expand during systole is labelled **compliance**. Compliance is directly related to elasticity of the vessels which tends to decrease with age. This is the mechanism underlying systolic hypertension which develops with ageing.
- **Diastolic blood pressure** is directly dependant on the amount of blood in the aorta during diastole which in turn is determined by **peripheral vascular resistance** and the **heart rate**. Increased diastolic pressure is caused by peripheral vasoconstriction, increase in blood viscosity as in leukaemias and polycythaemia and increase in heart rate. Factors that cause peripheral vasoconstriction by contracting the arteriolar smooth muscle include α-adrenergic stimuli (increased circulating catecholamines and angiotensin II, and increased total body sodium. **Excess sodium increases** plasma volume which increases systolic pressure and induces vasoconstriction of arterioles (increases **calcium-mediated contraction of the smooth muscle**).

Aetiological Classification of Hypertension

- Hypertension is classified into two types:
  - *Primary or essential or idiopathic (90–95%)*: Wherein cause of increased blood pressure is unknown.
  - *Secondary hypertension (5–10%)*: Hypertension secondary to diseases of kidneys, endocrines or other organs.
- Both primary and secondary hypertension can be benign or malignant:
  - ***Benign hypertension*** is characterized by a moderate elevation of blood pressure slowly rising over many years. Patients have a long and asymptomatic life, unless myocardial infarction or a cerebrovascular accident occurs.
  - ***Malignant hypertension*** is typically associated with a marked and rapid increase in blood pressure to 200/120 mm Hg or more. Patients present with papilloedema, retinal haemorrhages, hypertensive encephalopathy and renal failure. Life expectancy after diagnosis is generally less than 2 years, if not treated effectively.

Essential (Primary) Hypertension

- ***Genetic and environmental factors are both thought to play a role in its development.***
  - ***Genetic factors*:** Familial clustering and prevalence in twins has been observed, indicating a role of genetic factors.
  - ***Racial and environmental factors*:** Essential hypertension has been found to be more prevalent amongst blacks than in whites. Environmental factors like high salt intake, obesity, occupation (skilled population is more affected than unskilled), higher living standards and high levels of stress are also implicated.
- ***There are some risk factors which modify the course of essential hypertension. These include*:**
  - *Age*: Younger the age at which hypertension is seen, lower the life expectancy, particularly if it is left untreated.
  - *Sex*: Females with hypertension appear to do better than males.
  - *Atherosclerosis*: Hypertension increases the incidence of atherosclerosis-related complications.
  - *Other risk factors include* excess of alcohol intake and diabetes mellitus.

Secondary Hypertension

Secondary hypertension can result from conditions affecting different organs:

- **Renal causes**: Acute glomerulonephritis, chronic renal failure, polycystic disease, pyelonephritis, interstitial nephritis, amyloidosis, diabetic nephropathy and renin-producing tumours.
- **Endocrine causes**: Cushing syndrome, hyperaldosteronism, oral contraceptives, oestrogens, pregnancy induced, pheochromocytoma, acromegaly, primary hypothyroidism, thyrotoxicosis and hyperparathyroidism
- **Cardiovascular causes**: Coarctation of aorta (causes systolic hypertension in the upper part of the body) and polyarteritis nodosa
- **Neurological causes**: Acute stress, increased intracranial pressure and psychogenic
- **Miscellaneous causes**: Alcohol, obesity and pregnancy (preeclampsia)

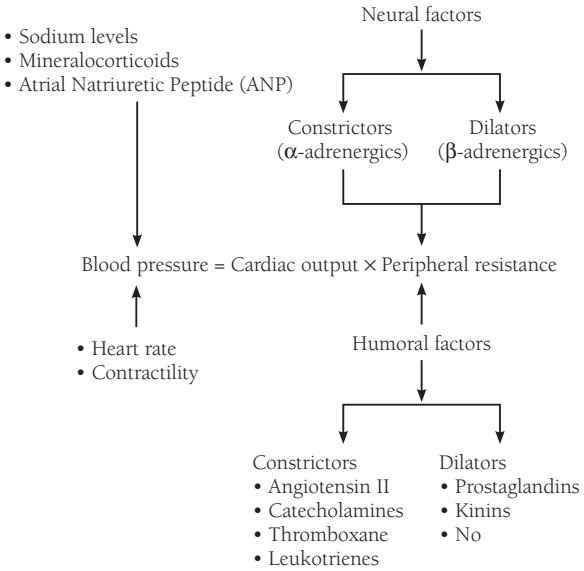
Renal Regulation of Blood Pressure

The kidneys and heart interact to regulate the vascular tone and blood volume by altering sodium balance. The various regulatory mechanisms involved are the following:

1. ***Activation of renin–angiotensin system*:** Renin is an enzyme that is produced by renal juxtaglomerular cells that are present in the proximity of afferent glomerular arterioles. It is released in response to decreased blood pressure in the afferent arterioles, increased levels of circulating catecholamines or low sodium levels. Renin cleaves angiotensinogen to angiotensin I, which undergoes peripheral catabolism to produce angiotensin II. Angiotensin II regulates blood pressure by:
   - Increasing vascular smooth muscle tone
   - Stimulating secretion of aldosterone
   - Regulating renal sodium and water resorption (reduction in GFR due to reduced blood flow results in increase in proximal tubular reabsorption of sodium)
2. ***Release of vasodepressor material*:** A number of vasodepressor materials and antihypertensives, eg, prostaglandins and nitrous oxide counter balance the vasopressor effect of angiotensin II.
3. ***Natriuretic factors*:** Atrial and ventricular myocardium secretes natriuretic peptides which inhibit the renin–angiotensin system thereby causing sodium excretion, diuresis and vasodilatation. Increased stretching of atria and ventricles of the heart due to increased BP induces release of natriuretic factors.

Role of Cardiac Output and Peripheral Resistance in Regulation of Blood Pressure

Role of cardiac output and peripheral resistance in regulation of blood pressure is depicted in Flowchart 10.5.



FLOWCHART 10.5. Role of cardiac output and peripheral resistance in regulation of blood pressure.

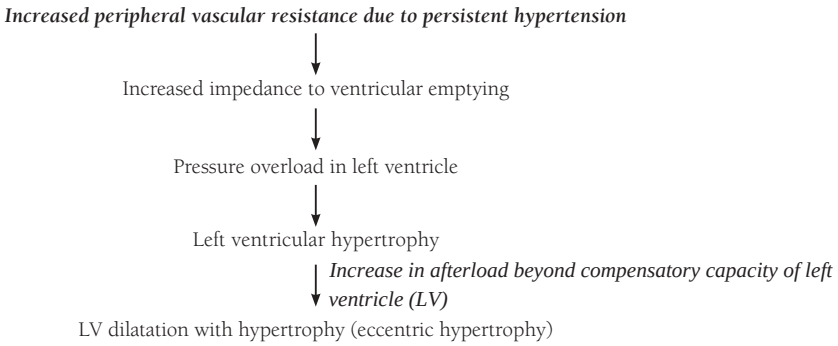
Effects of Hypertension

The major effects of systemic hypertension are noted in the following organs:
- Heart
- Blood vessels
- Kidneys
- CNS
- Retina

Effects on Heart (Hypertensive Heart Disease)

- Usually seen in association with systemic hypertension of prolonged duration, and is the second most common form of heart disease after IHD.
- Death in hypertensive patients is due to congestive heart failure, IHD, cerebrovascular accident (stroke) and renal failure.

Pathogenesis (*Flowchart 10.6*):



FLOWCHART 10.6. Pathogenesis of hypertensive heart disease.

Gross pathology: Marked hypertrophy of the heart, chiefly left ventricle
Microscopic findings: Enlargement and degeneration of myocardial fibres with areas of myocardial fibrosis

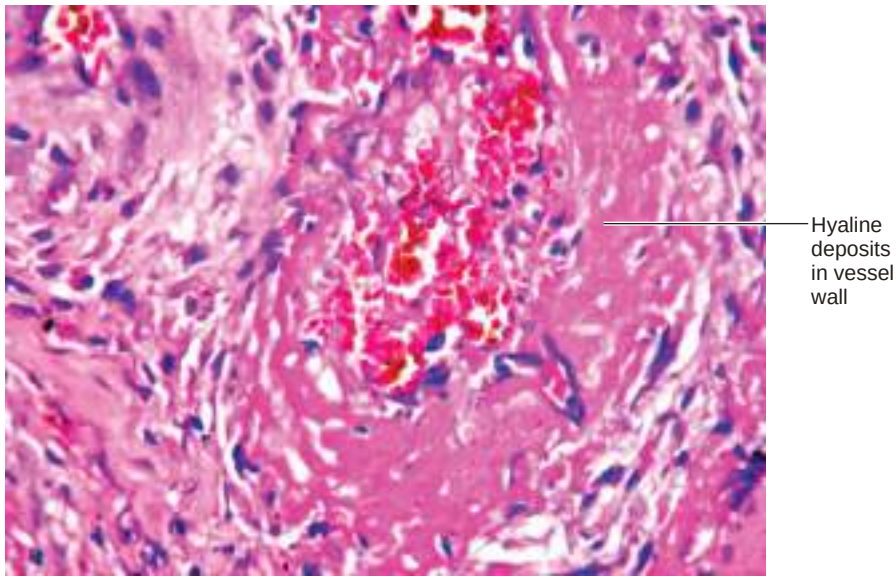


FIGURE 10.4. Hyaline arteriosclerosis showing thickening of the vessel wall and deposition of hyaline material (H&E; 100×).

Effects on Blood Vessels (Hypertensive Arteriolosclerosis)

Hypertension affecting blood vessels has three main pathological patterns, namely:

1. **Hyaline arteriosclerosis:**
   This pattern may be:
   - Physiological in origin when it occurs as a result of ageing.
   - Pathological in origin when it occurs due to hypertension or diabetes mellitus.

   ***Pathogenesis*:** Chronic haemodynamic stress of hypertension induces leakage of components of plasma and deposition of immunoglobulins, complement, fibrin and lipid in the vessel wall. In diabetes, nonenzymatic glycosylation of the basement membrane of small vessels makes them permeable to proteins, which leak through into the vessel wall to produce hyaline change.

   Pathology (Fig. 10.4): Vessel walls are thickened and lumina narrowed and eosinophilic hyaline material is deposited in the intima and media.
2. **Hyperplastic arteriolosclerosis:**
   This is usually a consequence of malignant hypertension or toxaemia of pregnancy.

   ***Pathogenesis*:** Increase in blood pressure causes endothelial injury which in turn leads to increased vascular permeability and leakage of plasma components. This is thought to stimulate smooth muscle proliferation and basement membrane duplication.

   ***Pathology*:** Vessels typically shows intimal thickening, which may manifest as:
   - *Onion skinning*—Concentric layers of hyperplastic intimal smooth muscle cells (Fig. 10.5)
   - *Mucinous intimal thickening*—Deposition of anhydrous ground salts
   - *Fibrous intimal thickening*—Laying down of collagen, elastic fibres and hyaline deposits in intima.
3. **Necrotizing arteriolitis:**
   This pattern of arteriosclerosis is typically associated with severe-or-malignant hypertension.

   ***Pathogenesis*:** Sudden elevation of pressure causes direct physical injury to vessel wall leading to endothelial damage with fibrin deposition and wall necrosis.

   ***Pathology*:** Hyaline sclerosis and fibrinoid necrosis of vessel wall, along with an infiltrate of neutrophils in adventitia.

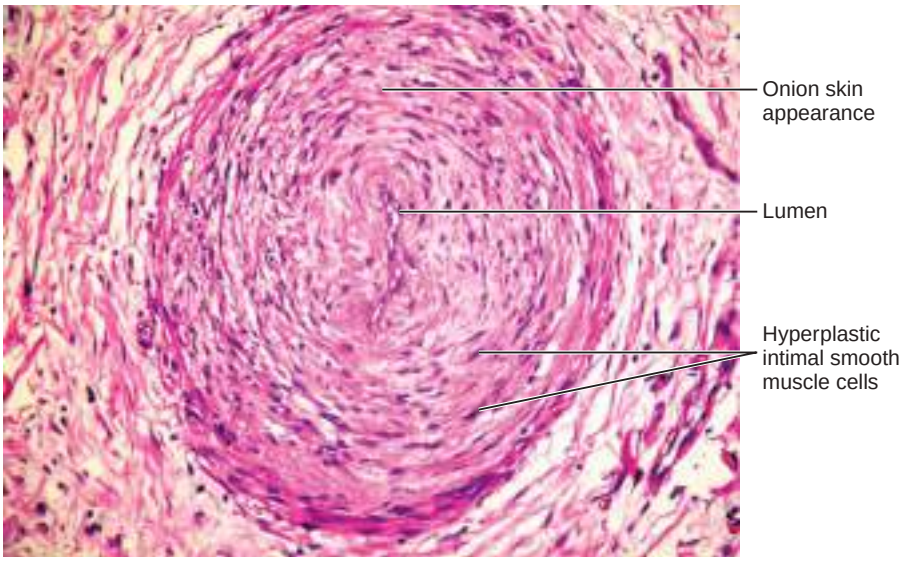


FIGURE 10.5. Hyperplastic arteriolosclerosis showing onion skinning (H&E; 100×).

Effects on Kidneys (Hypertensive Renal Disease)

Hypertensive renal disease may present as any of the following morphological patterns:

1. **Benign nephrosclerosis:** It is the spectrum of renal changes associated with the benign phase of hypertension. Benign nephrosclerosis is the most common form of renal disease in persons over 60 years of age (common autopsy finding), and its severity increases in the presence of diabetes mellitus.
   ***Clinical features*:**
   - Variable elevation of blood pressure
   - Headache and dizziness
   - Palpitations and nervousness
   - Renal function tests and urine examination may be normal in early stage; however, the patient may manifest with mild proteinuria and presence of hyaline and granular casts in the late stage.

   ***Gross pathology*:**
   - Both kidneys are reduced in size and weight due to cortical scarring (small contracted kidneys).
   - The capsule is adherent to cortical surface, which appears finely granular and resembles leather grain.

   ***Microscopic findings*:**
   - *Vascular changes*
     - *Hyaline arteriolosclerosis:* Homogeneous eosinophilic thickening (hyalinization) of the walls of small arteries and arterioles
     - *Intimal thickening*: Proliferation of smooth muscle cells in the intima of the arcuate and interlobular arteries along with medial hypertrophy and reduplication of internal elastic lamina
   - *Parenchymal changes*
     - Glomerular shrinkage
     - Deposition of collagen in Bowman's space
     - Periglomerular fibrosis and complete sclerosis of the glomerulus
     - Tubular atrophy and fine interstitial fibrosis
2. **Malignant nephrosclerosis:** A manifestation of malignant or accelerated hypertension, this pattern is uncommon and usually occurs as a superimposed complication in 5% cases of pre-existing benign hypertension; can occur in pure form also.

***Clinical features*:**
- Presents with headache, dizziness, impaired vision, papilloedema and deranged renal function
- Urine findings include haematuria and proteinuria

***Gross pathology*:**
- If superimposed on pre-existing benign hypertension, kidneys are small, shrunken and reduced in size.
- In the pure form, kidneys enlarge and show pin-point petechial haemorrhages on the cortical surface (**flea-bitten kidney**) due to rupture of arterioles and glomerular capillaries.

***Microscopic findings*:**
- *Necrotizing arteriolitis*: Fibrinoid necrosis, a few acute inflammatory cells and small haemorrhages
- *Hyperplastic intimal sclerosis or onion-skin proliferation*: Concentric laminar proliferation of smooth muscle cells, collagen and basement membrane material
- *Ischaemic changes*: Tubular loss, fine interstitial fibrosis and foci of infarction

Effects on CNS

- Stroke (cerebral haemorrhage and lacunar infarction)
- Carotid atheromas and transient ischaemic attacks
- Subarachnoid haemorrhage
- Hypertensive encephalopathy (neurological symptoms like disturbances in speech, vision, paraesthesias, fits and loss of consciousness)

Effects on Retina (Hypertensive Retinopathy)

- Focal spasm of the arterioles followed by progressive sclerosis (arteriolar walls become opaque with narrow light reflex)
- Chronic hypertension leads to intimal thickening, media wall hyperplasia and hyaline degeneration of arterioles.
- Severe hypertension causes necrosis of vascular smooth muscle and endothelial cells resulting in exudate formation ("soft exudates" are ill-defined and result from microinfarctions; whereas, "hard exudates" are due to leakage of protein from increased vessel permeability).
- Persistent increased pressure in the arterioles may result in formation of microaneurysms which may rupture leading to 'flame haemorrhages'.
- Impeded arteriolar circulation results in a compression of venules and ultimately dilatation as arteriole and venous basement membranes are adherent with shared collagen fibres at the crossing points.
- Development of a depression in the wall of the venule (arteriovenous nicking)
- Papilloedema (swelling of the optic disk)

Keith–Wagener–Barker classification of hypertensive retinopathy

Grade I: focal narrowing of the arterioles, mild arteriovenous nicking
Grade II: arteriole narrowing, copper wiring present, arteriovenous nicking more accentuated
Grade III: arteriole narrowing, silver wiring present, haemorrhages, soft and hard exudates, disappearance of the vein under the arteriole, disk normal
Grade IV: arterioles are fine fibrous cords; same as grade III except papilloedema is present

Laboratory Work-Up of Essential Hypertension and Its Consequences

- Heart disease, eg, ECG, chest X-ray for cardiomegaly and ECHO for left ventricular hypertrophy
- Renal disease, eg, urine analysis, serum blood urea nitrogen (BUN), creatinine, renal ultrasound and angiography
- Mineralocorticoid excess states, eg, serum electrolytes
- Pheochromocytoma, eg, urinary catecholamines
- Diabetes mellitus, eg, serum glucose
- Lipid abnormalities, eg, lipid profile

Q. Classify vascular tumours.

Ans. ***Classification***

1. **Benign neoplasms, developmental and acquired conditions**
   - (a) Haemangioma
     - (i) Capillary haemangioma
     - (ii) Cavernous haemangioma
     - (iii) Pyogenic granuloma
   - (b) Lymphangioma
     - (i) Simple (capillary lymphangioma)
     - (ii) Cavernous lymphangioma (cystic lymphangioma)
   - (c) Glomus tumour
   - (d) Vascular ectasias
     - (i) Nevus flammeus
     - (ii) Spider telangiectasia (arterial spider)
     - (iii) Hereditary haemorrhagic telangiectasia (Osler–Weber–Rendu disease)
   - (e) Reactive vascular proliferations
     Bacillary angiomatosis
2. **Intermediate grade neoplasms**
   - (a) Kaposi sarcoma
   - (b) Hemangioendothelioma
3. **Malignant neoplasms**
   - (a) Angiosarcoma
   - (b) Hemangiopericytoma

Q. Describe the clinicopathological features of haemangiomas.

Ans. Haemangiomas are common benign tumours of infancy and childhood. These are difficult to differentiate from malformations or hamartomas.

- May be → Localized (angiomas)
  → Diffuse (angiomatosis; involve large segments of the body, eg, entire extremity)
- May be → Superficial (head and neck)
  → Internal or visceral (liver)

1. **Capillary haemangioma**
   - (a) Largest single type of vascular tumour believed to be a proliferation of vascular endothelial cells. It is composed of capillary channels with RBCs.
   - (b) Most commonly found in the skin, subcutaneous tissue and mucous membranes of the oral cavities and lips; presents as a red to reddish-purple raised lesion. May also be seen in the liver, spleen and kidneys.
   - (c) **'Strawberry type'** of capillary haemangioma is very common and tends to regress by seven years of age in 75–90% cases.
   - (d) Capillary haemangioma may cause cosmetic disturbance or manifest with bleeding due to traumatic ulceration.
2. **Pyogenic granuloma (lobular capillary haemangioma or polypoid capillary haemangioma)**
   - (a) Typically presents as a red/pink to purple nodule, smooth or lobulated, which may follow trauma.
   - (b) Shows a striking resemblance to exuberant granulation tissue with oedema and acute and chronic inflammation.
   - (c) **'Granuloma gravidarum'** is a pyogenic granuloma occurring in 10% of the pregnant women (regresses after delivery).
3. **Cavernous haemangioma**
   - (a) Usually involves deeper structures
   - (b) Locally destructive large lesions, which show no tendency to regress
   - (c) Red-blue, soft, spongy 1–2 cm in diameter
   - (d) Rare giant forms that affect large subcutaneous areas of face or extremities

(e) Sections show a poorly defined unencapsulated lesion, composed of large cavernous vascular spaces separated by scant connective tissue stroma.

Q. Describe the clinicopathological features of lymphangiomas.

Ans. Lymphangioma is a benign lymphatic counterpart of haemangioma. It is of two types:

1. **Capillary lymphangioma** (also called **lymphangiomacircumscriptum)**
   (a) It is millimetres to centimetres in diameter.
   (b) Tends to occur subcutaneously in head and neck region and axilla.
   (c) Is distinguished from capillary channels by absence of blood cells.
   (d) Lobulated but unencapsulated aggregates of thin-walled lymphatics, separated by scant connective tissue stroma comprises the lesion.
2. **Cavernous lymphangioma** (also called **cystic hygroma)**
   (a) Occurs in children in neck and axilla and rarely retroperitoneal region
   (b) Histopathology shows massively dilated cystic lymphatic spaces lined by endothelial cells and separated by connective tissue stroma that often contains lymphoid aggregates.
   (c) Margins not well-defined and therefore difficult to remove

Q. Describe the clinicopathological features of glomus tumour.

Ans. Glomus tumour (glomangioma) is an exquisitely painful tumour arising from modified smooth muscle cells of the glomus body (a specialized arteriovenous anastomosis involved in thermoregulation).

- Manifests as a small (<1 cm in diameter), red-blue firm nodule usually located in the distal portion of digits.
- Histopathology shows aggregates and nests of specialized glomus cells (small round to oval with scanty cytoplasm) lying in connective tissue stroma containing branching vascular structures.

Q. Enumerate the intermediate grade (borderline or low-grade malignant) vascular tumours and write briefly about them.

Ans. Intermediate grade vascular neoplasms include

1. **Kaposi sarcoma (KS):** It was first described by Kaposi in 1872 and is frequently associated with AIDS. There are four known forms of the disease:
   (a) **Chronic/Classic/European KS**
      (i) Mostly affects older men of Eastern European or Mediterranean descent
      (ii) Not associated with HIV
      (iii) Presents with multiple red to purple skin plaques or nodules on extremities
      (iv) Viscera and mucosa are involved in 10% cases
   (b) **Lymphadenopathic/African/Endemic KS**
      (i) Particularly prevalent among young Bantu children of South Africa
      (ii) Presents with localized or generalized lymphadenopathy
      (iii) Disease course is aggressive and there is a strong association with AIDS
   (c) Transplant (immunosuppression)-associated KS
      (i) Occurs several months to a few years posttransplant in patients receiving high doses of chemotherapy
      (ii) Aggressive; involves lymph nodes, mucosa and visceral organs (usually fatal)
      (iii) Skin lesions may be absent
   (d) AIDS-associated KS
      (i) KS is the most common AIDS-associated cancer in the United States.
      (ii) Involvement of lymph nodes and the gut and wide and early dissemination is the hallmark of the disease.
      (iii) Most patients, however succumb to AIDS-associated opportunistic infections rather than consequences of KS.

***Morphology of KS*:** Three stages are identified:

- **Patch stage:** Pink to red, solitary to multiple macules in the distal lower extremities

***Microscopy*:** Dilated, irregular and angulated blood vessels lined by endothelial cells with an interspersed infiltrate of lymphocytes, plasma cells and macrophages

- **Plaque stage:** Patch lesion over time converts into larger, violaceous and raised plaques

***Microscopy*:** Dilated, jagged and dermal vascular channels lined by plump cells accompanied by perivascular aggregates of spindle cells; scattered between vascular channels are haemosiderin-laden macrophages, lymphocytes, plasma cells and pink hyaline globules of uncertain origin

- **Nodular stage:** At a later stage, lesions become distinctly nodular and may be accompanied by involvement of lymph nodes and viscera.

***Microscopy*:**

- Sheets of plump proliferating spindle cells in the dermis and subcutaneous tissue
- Small scattered slit-like vessels in a background containing RBCs and pink droplets
- Marked haemorrhage, haemosiderin-laden macrophages and lymphocytes

Pathogenesis of KS

- Ninety-five percent of KS lesions are infected with KSHV (KS-associated herpes virus called human herpes virus) or HHV-8.
- Immunosuppression is an important cofactor in pathogenesis and clinical expression of the disease.
- KSHV proteins disrupt the control of cellular proliferation and prevent apoptosis of endothelial cells, through the production of P53 inhibitors and a viral homologue of cyclin D.

2. **Hemangioendothelioma**
   (a) Group of vascular neoplasms showing histological features intermediate between benign haemangioma and frankly malignant angiosarcomas.
   (b) A representative of this group is epithelioid hemangioendothelioma. It occurs in medium-sized and large veins in soft tissue (well-defined vascular channels are conspicuous and tumour cells are plump epithelial like).

Q. Enumerate the malignant vascular tumours and write briefly about them.

Ans. ***Malignant vascular tumours are of two main types*:**

1. **Hemangiopericytoma**
   (a) Heterogeneous group of neoplasms with a fleshy or spongy consistency and thin-walled branching, staghorn vascular pattern. It is derived from 'pericytes'.
   (b) Two-thirds of these tumours have a benign course; one-third are malignant.
   (c) Presence of necrosis, high mitotic rate and nuclear pleomorphism are associated with aggressive behaviour.
2. **Angiosarcoma**
   (a) Malignant vascular tumour derived from endothelium.
   (b) Angiosarcomas vary from highly differentiated tumours to those resembling epithelial neoplasms like carcinomas and melanomas.
   (c) Stain positive for CD31, CD34 or VW factor.
   (d) Seen in older adults; most commonly in skin, soft tissue, breast and liver.
   (e) May arise in the setting of lymphoedema, radiation exposure or foreign material introduced into the body iatrogenically or accidentally.
   (f) Local invasion and distal metastatic spread is common. Outcome is poor with very few surviving 5 years.

11

Disorders of the Heart

Normal Heart

- The weight of a **normal heart** averages approximately 250–300 g in females and 300–350 g in males.
- It is enclosed in a double–walled sac called **pericardium**. The pericardium consists of an outer fibrous layer called the parietal pericardium and an inner layer called the visceral pericardium or the epicardium.
- The wall of the human heart is composed of three layers. The outer layer is the **epicardium**; the middle layer is called **myocardium** and the innermost layer is called **endocardium.**
- The **endocardium** merges with the endothelium, which lines the blood vessels and covers heart valves (valvular endocardium). The usual thickness of free wall of right ventricle is 0.3–0.5 cm and that of the left ventricle is 1.3–1.5 cm.
- Structure of the normal heart is depicted in (Fig. 11.1). An increase in cardiac weight or size is termed *cardiomegaly.* Thickening of ventricular wall is called *hypertrophy*, and an enlarged chamber size indicates *dilation.*
- The *myocardium* is composed of specialized muscle cells called *cardiac myocytes.* The basic contractile unit of cardiac muscle is called the *sarcomere,* which is composed of thick and thin filaments containing *myosin* and *actin*, respectively, along with regulatory proteins troponin and tropomyosin. The striated appearance of cardiac myocytes is due to a specific arrangement of sarcomeres. The sliding of the actin filaments between the myosin filaments towards the centre of each sarcomere is the mechanism responsible for the contractility of the cardiac muscle.
- Besides myocytes other cells that are present in heart include endothelial cells and fibroblasts. Cardiac myocytes contain structures called *intercalated disks* that join individual cells to allow mechanical and electrical coupling.

Vascular Supply of the Heart (Fig. 11.2)

The three major epicardial coronary arteries that perfuse the heart are

(1) Anterior descending branch of the left coronary artery (LAD), which supplies most of apex of the heart, the anterior wall of the left ventricle and the anterior two-thirds of the ventricular septum.
(2) Circumflex branch of the left coronary artery (LCX) gives rise to posterior descending branch (PDA) and thereby perfuses the posterior third of the septum.
(3) Right coronary artery (RCA) which supplies the right atrium, right ventricle, interventricular septum and SA and AV nodes.

Majority of the perfusion of the myocardium by the coronary arteries occurs during ventricular diastole, when there is no compression of the cardiac microcirculation due to cardiac contraction.

Q. Enumerate the types of heart disease.

Ans. There are five major types of heart disease:

- Ischaemic heart disease (IHD)
- Hypertensive (systemic/pulmonary) heart disease
- Nonischaemic primary myocardial disease

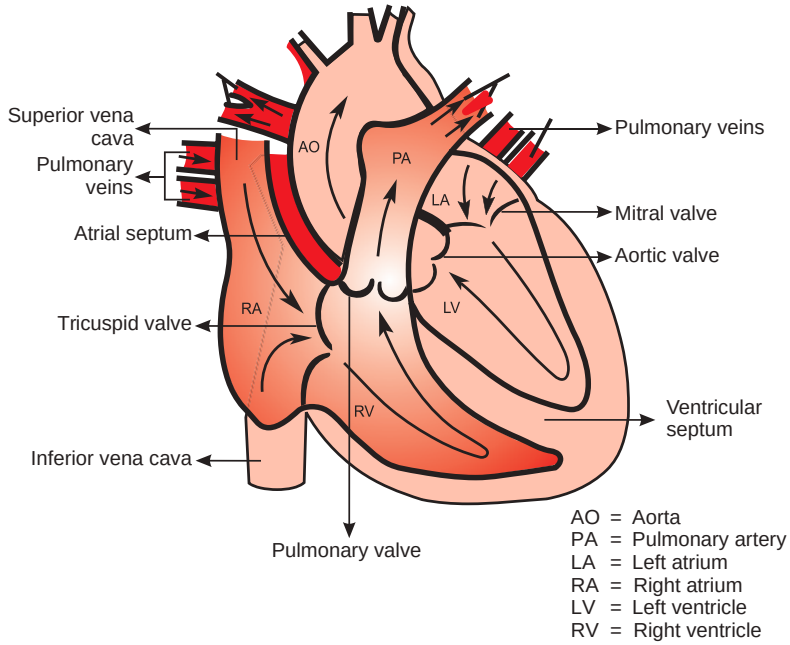


FIGURE 11.1. Pictorial representation of normal structure of heart.

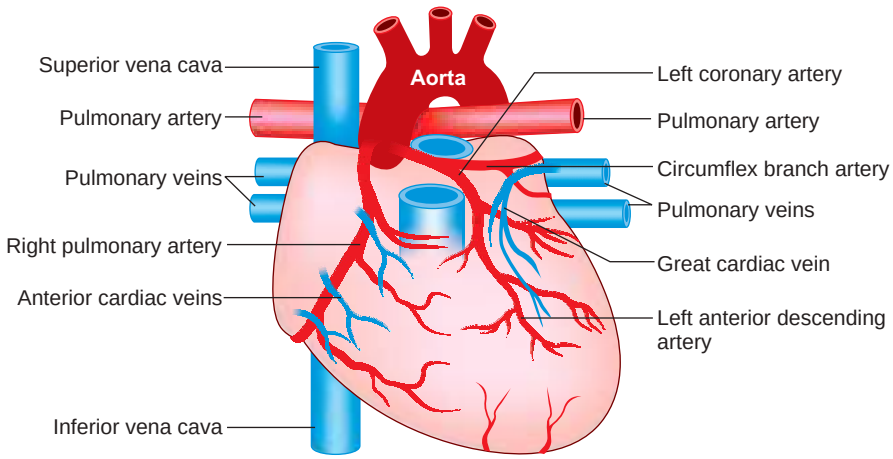


FIGURE 11.2. Vascular supply of the heart.

- Congenital heart disease
- Valvular heart disease

Q. Define IHD. Enumerate the clinical syndromes associated with IHD.

Ans. IHD is a term for a group of closely related syndromes resulting from myocardial ischaemia.

- Ischaemia is more harmful than 'isolated hypoxaemia' (insufficiency of O_2) and is characterized by:
  - Insufficiency of O_2
  - Decreased availability of nutrients
  - Decreased removal of metabolites
- **Coronary atherosclerosis** accounts for myocardial ischaemia in more than 90% cases of IHD; therefore, IHD is also called **coronary artery disease (CAD)** or **coronary heart disease (CHD)**.

Clinical syndromes associated with IHD are

1. Myocardial infarction (MI)
2. Angina pectoris:
   (a) Stable
   (b) Prinzmetal or variant
   (c) Unstable or crescendo
3. Chronic ischaemic heart disease with heart failure
4. Sudden cardiac death

These clinical syndromes are a result of a complex dynamic interaction between

- Fixed atherosclerotic narrowing
- Intraluminal thrombosis overlying a disrupted atherosclerotic plaque
- Platelet aggregation and vasospasm

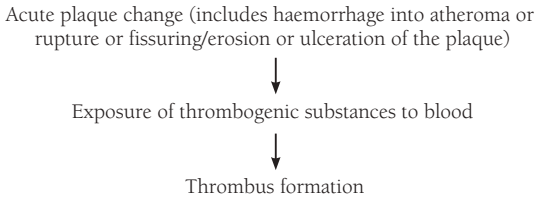
Q. Write briefly on the role of fixed coronary obstruction in the pathogenesis of IHD.

Ans. Ninety percent patients with IHD have underlying atherosclerosis with presence of solitary or multiple lesions, causing **at least 75% reduction of cross-sectional area of at least one major artery**.

- Common locations for clinically significant stenosis include the first several cm of LAD (left anterior descending artery) and LCX (left circumflex artery), and the entire length of RCA (right coronary artery).
- Usually 2 or all 3 arteries (LAD, LCX and RCA) are involved.
- Major secondary epicardial branches may also be involved but atherosclerosis of intramural branches is rare.

Q. Write briefly on the role of acute plaque change in the pathogenesis of IHD.

Ans. Role of acute plaque change in the pathogenesis of IHD (Flowchart 11.1):



FLOWCHART 11.1. Role of acute plaque change in the pathogenesis of IHD.

Factors that trigger/contribute to acute plaque alterations:

- Adrenergic stimulation
- Structure and composition of plaque (eccentric location, large soft core and thin fibrous cap predispose to plaque alterations)
- Most dangerous lesions are the moderately stenotic (50–60% stenosis) lipid-rich atheromas (plaques causing >60% obstruction reduce blood flow; thus, decreasing mechanical stress in the vessel wall, reducing chances of its disruption. Slowly developing occlusions even if they are high grade, are less dangerous because they stimulate collateral vessel formation)

Q. Write briefly on the role of coronary thrombosis in the pathogenesis of IHD.

Ans. Acute transmural MI is usually caused by superimposition of a thrombus on a disrupted, previously partially stenotic plaque, causing total occlusion.

Thrombus formation is aided by:

- Thromboxane A_2 and other platelet constituents
- Fibrinogen
- Lipoprotein (a), which inhibits fibrinolysis

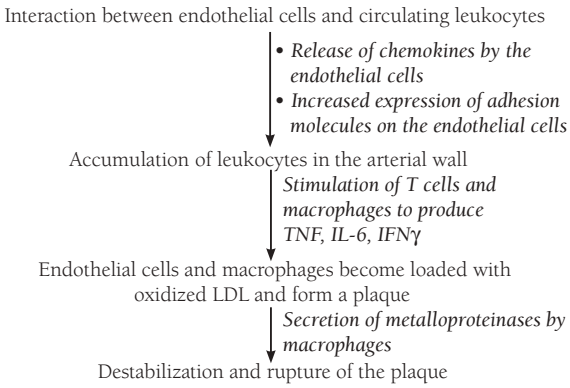
Q. Write briefly on the role of vasoconstriction in the pathogenesis of IHD.

Ans. Vasoconstriction/spasm may contribute to the pathogenesis of prinzmetal angina as well as acute MI. It is induced by:

- Circulating adrenergics
- Release of platelet contents
- Impaired secretion of endothelial cell relaxing factors, eg, nitrous oxide
- Release of mediators from perivascular cells like mast cells

Q. Write briefly on the role of inflammation in the pathogenesis of IHD?

Ans. Role of inflammation in IHD (Flowchart 11.2):



FLOWCHART 11.2. Role of inflammation in IHD.

Note: Inflammation is thought to have an established role in the pathogenesis of atherosclerosis and proteins involved in inflammation are considered potential markers of CAD, eg, CRP (C-reactive protein).

Q. Define and classify angina pectoris. Write briefly on the different types of angina.

Ans. Angina pectoris is defined as a symptom complex of IHD characterized by paroxysmal recurrent attacks of substernal or precordial chest discomfort (constricting, squeezing and choking, knife like) caused by transient (from 15 s to 15 min) of myocardial ischaemia that falls short of inducing cellular necrosis that defines infarction.

Three Overlapping Patterns

1. **Stable/exertional/classical angina**
   (a) Reduction of coronary perfusion due to chronic stenosing coronary atherosclerosis
   (b) Heart is vulnerable to ischaemia whenever there is increased demand, ie, physical activity and emotional excitement
   (c) Relieved by rest/decreased demand and nitroglycerin (decreases cardiac work by dilating peripheral vasculature)

2. **Prinzmetal variant angina**
   (a) Uncommon pattern of episodic angina that occurs at rest and is due to coronary artery spasm
   (b) Attacks unrelated to physical activity, heart rate or blood pressure
   (c) Elevation of ST segment (indicative of transmural ischaemia) is typically seen
   (d) Responds promptly to vasodilators like nitroglycerin and calcium channel blockers
3. **Unstable/crescendo angina**
   (a) Repeated episodes of pain with progressively increasing (crescendo) frequency
   (b) Often occurs at rest and tends to be of prolonged duration
   (c) Induced by disruption of an atherosclerotic plaque with superimposed partial thrombosis and embolization/vasospasm or both
   (d) Precedes acute MI in many patients (also called preinfarction angina)

Q. Differentiate among stable angina, Prinzmetal variant angina and unstable/crescendo angina.

Ans. Differences among stable angina, Prinzmetal variant angina and unstable/crescendo angina are summarized in Table 11.1.

TABLE 11.1. Differences among stable angina, Prinzmetal variant angina and unstable/crescendo angina

Features	Stable angina	Prinzmetal variant angina	Unstable/ crescendo angina
Cause	Fixed coronary atherosclerotic narrowing	Due to coronary artery spasm	Induced by disruption of an atherosclerotic plaque with superimposed partial thrombosis and embolization/vasospasm or both (dynamic stenosis)
Precipitating factors	Heart vulnerable to ischaemia whenever increased demand, ie, physical activity and emotional excitement	Occurs at rest, not related to physical activity or emotional excitement	Often occurs at rest and tends to be of prolonged duration
Relieving factors	Relieved by rest/decreased demand and nitroglycerin (decreases cardiac work by dilating peripheral vasculature)	Responds promptly to vasodilators like nitroglycerin and calcium channel blockers	May respond to vasodilators like nitroglycerin and calcium channel blockers
Outcome	Responds to medication	Transmural ischaemia, which generally responds to medication	Harbinger of subsequent acute MI in many patients (also called preinfarction angina)

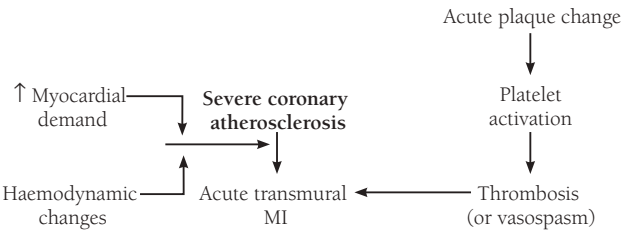
Q. What is myocardial infarction (MI)? Write briefly on the aetiopathogenesis, clinical features and morphological evolution of an acute MI.

Ans. MI is defined as myocardial ischaemia that induces cellular necrosis. It is a leading cause of death in industrialized nations.

Incidence and Risk Factors

- Ten percent infarcts occur in patients <40 years and 45% in patients <65 years (increasing risk with increasing age)
- Males are more commonly affected than females (the latter show increasing risk with decreasing oestrogen levels)
- Hypertension, diabetes mellitus, hyperlipoproteinaemias, increased apolipoprotein B, increased lipoprotein (a), increased C-reactive protein and hyperhomocystinuria are established risk factors for acute MI

Evolution of Coronary Arterial Occlusion (Flowchart 11.3)



FLOWCHART 11.3. Evolution of coronary arterial occlusion.

- **In 10% cases, MI is not associated with atherosclerosis and is caused by other mechanisms:**
  1. **Vasospasm:** Intense, relatively prolonged, vasospasm with or without coronary atherosclerosis and platelet aggregation can induce acute MI
  2. **Emboli:** May arise from left atrium due to atrial fibrillation, left-sided mural thrombosis, vegetative endocarditis, paradoxical embolus from right side of heart or peripheral veins
  3. **Unexplained:** In one-third patients, small intramural coronary vessel disease (like vasculitis) or haematological abnormalities, eg, haemoglobinopathies, may lead to acute coronary episodes

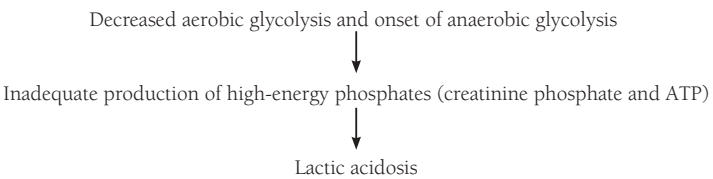
Clinical Features of Acute MI

- Squeezing, constricting or burning type of retrosternal chest pain which most often occurs in the early morning hours (attributed to the increase in catecholamine-induced platelet aggregation and increased serum concentrations of plasminogen activator inhibitor-1 post awakening). The pain may radiate up to the neck, shoulder and jaw and down to the ulnar aspect of the left arm.
- Dyspnoea due to pulmonary congestion/pulmonary oedema or impaired contractility of the heart
- Indigestion, feeling of fullness and gas
- Apprehension or anxiety
- Excessive sweating
- Nausea with or without vomiting
- Light headedness with or without syncope
- Cough or wheezing
- Hiccupping (which is thought to be due to irritation of the phrenic nerve or diaphragm)
- Rapid thready pulse
- May be asymptomatic, discovered on ECG (silent MIs are common in underlying diabetes mellitus and elderly patients)

Myocardial Response

Decreased blood supply induces profound functional, biochemical and morphologic changes.

- ***Biochemical consequences*** **(**Flowchart 11.4**)**



FLOWCHART 11.4. Biochemical consequences of acute MI.

- ***Morphological events***
  - ATP depletion leads to loss of contractility within a few minutes.
  - A state of irreversible injury sets in within 20–40 min.
  - Microvascular injury begins within 1 h.

The sequential morphologic changes in acute MI are summarized in Table 11.2.

TABLE 11.2. Morphologic changes in acute MI

Time	Gross changes	Microscopic changes
0–4 h	None	Waviness of fibres at border
4–12 h	None (the infarcted area can be highlighted by immersion of the dead tissue in triphenyl tetrazolium chloride, which gives a brick red colour to intact areas which have lactate dehydrogenase activity and do not stain the infracted area as the enzymes have leaked out in the latter due to membrane injury).	Beginning of coagulation necrosis, oedema
12–24 h	Dark mottling	Coagulative necrosis with pyknosis of nuclei; neutrophilic infiltration, myocyte hypereosinophilia; marginal contraction band necrosis
1–3 days	Hyperaemia around a yellow-tan infarct centre	Disintegration of dead myofibrils followed by phagocytosis of dead cells by macrophages at infarct border
3–7 days	Maximally yellow-tan and soft; well-delineated hyperaemic border	Early formation of fibrovascular granulation tissue at margins
7–10 days	Red-grey depressed infarct borders	Well-established granulation tissue with new blood vessels and collagen deposition
10–14 days	Progressive formation of a grey-white scar	Increased collagen deposition with decreased cellularity
2–8 weeks	Scarring complete	Dense collagenous scar forms

Q. Write briefly on reperfusion injury.

Ans. Infarct modification by reperfusion (restoration of blood flow by thrombolysis, percutaneous intervention and bypass surgery):

- Reperfusion within 15–20 min revives everything and prevents all necrosis.
- Thrombolysis by tissue plasminogen activator/streptokinase reestablishes blood flow/rescues ischaemic (and not dead) myocardium when given within first 3–4 h. Anticoagulant therapy with heparin, thrombin inhibitors and factor Xa inhibitors are used to prevent clot propagation. PTCA (percutaneous transluminal coronary angioplasty) relieves some of the obstruction caused by the plaque as well.
- Reperfusion can sometimes trigger deleterious effects labelled 'reperfusion injury', which could manifest as
  - **Arrhythmias:** Due to unstable myocardium.
  - **Haemorrhagic infarct:** A partially completed and reperfused infarct is haemorrhagic (vasculature injured due to ischaemia becomes leaky when the flow is restored).
  - **Contraction bands:** Intensely eosinophilic transverse bands composed of closely packed hypercontracted sarcomeres (produced by exaggerated contraction of myofibrils due to exposure to high concentration of calcium ions at the instant perfusion is reestablished).
  - **Stunned myocardium:** Refers to the persistence of biochemical abnormalities for days to several weeks after rescue from ischaemia by reperfusion.

Q. Enumerate the types of myocardial infarcts. Differentiate between transmural and subendocardial infarcts.

Ans. Depending on the thickness of the myocardium involved, myocardial infarcts are classified into:

1. Transmural infarcts: Involve the whole thickness of the ventricular wall in the distribution of a single coronary artery
2. Subendocardial infarcts: Involve only the inner one-third to one-half of the ventricular thickness
3. Multifocal microinfarcts: Multifocal microinfarction is seen when small intramural vessels are involved by vasculitis, microembolization or vasospasm.

The differentiating features between transmural and subendocardial infarcts are enlisted in Table 11.3.

TABLE 11.3. Differences between transmural and subendocardial infarcts

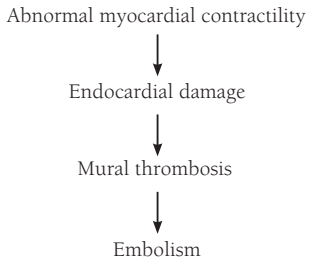
Features	Transmural infarct	Subendocardial infarct
Extent	Involves the whole thickness of the ventricular wall in the distribution of a single coronary artery	Involves only the inner one-third to one half of the ventricular thickness. May be multifocal and has a circumferential distribution.
Frequency	More common (95%)	Less common (5%)
Causes	Associated with coronary atherosclerosis, acute plaque change, superimposed completely obstructive thrombosis	No plaque disruption seen
Epicarditis	Common	Not common
Cardiac aneurysm formation	May be seen	Not seen
ECG changes	Elevation of ST segment	No ST elevation

Q. Enumerate the consequences and complications of acute MI.

Ans. Most deaths occur within one hour of onset of an acute MI. Three-fourth patients have one or more complications. Complications of acute MI include:

1. **Left ventricular contractile dysfunction:** Abnormality in left ventricular function is proportionate to the size of the infarct and may result in:
   (a) Left ventricular failure with hypotension and pulmonary vascular congestion.
   (b) Pump failure (cardiogenic shock; seen in 10–15% cases. Caused by a large infarct involving more than 40% of left ventricle area and is associated with a 70% mortality rate).
2. **Arrhythmias:**
   (a) Conduction disturbances due to myocardial irritability (sinus tachycardia, bradycardia, ventricular premature contractions, ventricular tachycardia, ventricular fibrillation and asystole)
   (b) Infarcts of inferoseptal region (area lodging bundle of His) are associated with heart block.
3. **Myocardial rupture:**
   (a) Myocardial rupture (due to transmural necrosis) may cause haemopericardium and cardiac tamponade
   (b) Complete rupture of ventricular wall/septum leads to formation of a left to right shunt
   (c) Incomplete rupture leads to formation of a pseudoaneurysm.
   (d) Papillary muscle rupture (most common 3–7 days after onset of infarct) causes valvular dysfunction (mitral regurgitation)
4. **Pericarditis:** Could be **early pericarditis (fibrinous or fibrinohaemorrhagic)** or **delayed immunologically mediated pericarditis (Dressler syndrome** which is seen 2–10 weeks after infarction)
5. **Right ventricular infarction:** Isolated right ventricular infarction is rare. Usually accompanies ischaemic injury of left ventricle and septum

6. **Infarct extension/expansion:** New necrosis/weakening and disproportionate stretching, thinning or dilatation of infarct, leads to its extension/expansion
7. **Embolism from mural thrombosis (**Flowchart 11.5**):** Abnormal myocardial contractility leads to endocardial damage which in turn leads to mural thrombosis and embolism



FLOWCHART 11.5. Pathogenesis of embolization from mural thrombosis.

8. **Ventricular aneurysm formation:**
   (a) Late complication
   (b) Associated with a large transmural anteroseptal infarct that converts into thin scar tissue
9. **Papillary muscle dysfunction:** Postinfarct mitral regurgitation due to ischaemic injury to papillary muscle and underlying myocardium; may later lead to papillary muscle fibrosis.
10. **Progressive late heart failure:** Chronic ischaemic heart disease (also called **ischaemic cardiomyopathy**) is caused by postinfarction cardiac decompensation due to exhaustion of compensatory hypertrophy of noninfarcted myocardium.

Complications occurring within first 72 h include cardiogenic shock, arrhythmias, acute pulmonary oedema and cardiac tamponade. Late complications include cardiac aneurysm formation, Chronic IHD or ischaemic cardiomyopathy, congestive heart failure, pulmonary hypertension and delayed pericarditis.

Q. Write briefly on the laboratory diagnosis of acute myocardial infarction (MI).

Ans. A patient is diagnosed with myocardial infarction if two (probable) or three (definite) of the following WHO criteria are met with:

- Clinical history of ischaemic type of chest pain lasting for more than 20 min.
- Changes in serial ECG tracings such as ST elevation/inverted T wave/appearance of Q wave.
- Rise in levels of serum cardiac biomarkers or enzymes, which leak out of the damaged myocardium into the blood, such as:

1. Creatinine kinase (CK)
   (a) **Different isoenzymes of CK include MM** (from skeletal muscle and heart), **MB** (principally from myocardium, particularly MB2) and **BB** (from brain and lung).
   (b) **CK activity**: Begins rising in 2–4 h, peaks in 24 h and falls in 72 h.
   (c) **CKMB**: More specific/begins rising in 4–8 h, peaks in 18 h and falls in 48–72 h.
   (d) **CKMB2/CKMBI ratio >1.5 is a highly sensitive indicator of myocardial injury.**

2. Troponins (Tn)
   (a) Troponins are proteins that regulate calcium mediated contraction of cardiac and skeletal muscle.
   (b) Two types, namely, TnI and TnT
   (c) Not normally detectable in serum; elevated in acute MI
   (d) Troponins of different origins can be distinguished by specific antibodies, which can also be used for quantitative assays
   (e) **Most sensitive and specific cardiac markers; as sensitive as CKMB and more specific**

(f) Troponin levels remain elevated for 7–10 days permitting a late diagnosis/evaluation of progression of infarct.

3. **Lactate dehydrogenase (LDH)**
   (a) LDH1 is myocardium specific
   (b) $\frac{\text{LDH1}}{\text{LDH2}} > 1$ helps in diagnosis of acute MI
   (c) Rises after 24 h, reaches a peak in 3–6 days and returns to normal in 14 days.
4. **Myoglobin**
   (a) **First cardiac marker to become elevated**
   (b) Lacks cardiac specificity
   (c) Excreted rapidly in the urine
   (d) Returns to normal within 24 h of the initiation of MI

- ***Other investigations***
  - Echocardiogram (to see abnormalities of regional wall motion)
  - Radioisotopic studies (radionuclide scan)
  - Perfusion scintigraphy (for regional perfusion)
  - MRI (for structural characterization)

Q. Write briefly on Dressler syndrome.

Ans. Dressler syndrome (also called postmyocardial infarction syndrome):

- Thought to be an autoimmune reaction to necrotic muscle
- Occurs weeks or months after infarction
- Presents with fever, pericarditis and pleurisy
- Treated with aspirin and NSAIDs or corticosteroids

Q. Define sudden cardiac death.

Ans. Sudden cardiac death is defined as unexpected death from cardiac cause within one hour of onset of symptoms. It may be a consequence of:

- IHD
- Congenital structural abnormality of heart and blood vessels (aortic valve stenosis/mitral valve prolapse)
- Myocarditis
- Pulmonary hypertension
- Abnormality of cardiac conduction system
- Isolated hypertrophy/increased cardiac mass

Q. Define congenital heart disease (CHD). Write briefly on its aetiopathogenesis.

Ans. CHD is defined as an abnormality of heart and blood vessels which is present since birth and due to faulty embryogenesis during third to eighth gestational weeks (when major cardiovascular structures develop). May result in:

- Severe anomalies, which are incompatible with life
- Less severe anomalies → May manifest soon after birth (at the time of change over from fetal to postnatal circulation)
  → May manifest in adulthood

Incidence

- 6–8/1000 live-born full-term infants
- Incidence higher in premature infants and stillborns

Aetiopathogenesis

Both genetic factors and maternal risks are implicated.

Genetic Factors

- ***Single gene mutations*** (genes encoding transcription factors required for normal cardiac development, eg, GATA4, Tbx5 and genes encoding components of Notch pathway)
- ***Small chromosomal deletions,*** eg, deletion of chromosome 22q11.2 in DiGeorge syndrome, which leads to abnormal development of 4th bronchial arch and derivatives of 3rd and 4th pharyngeal pouches responsible for the development of heart.
- ***Additions and deletions of whole chromosomes,*** eg, trisomy of chromosomes13, 15, 18 and 21, and Turner syndrome

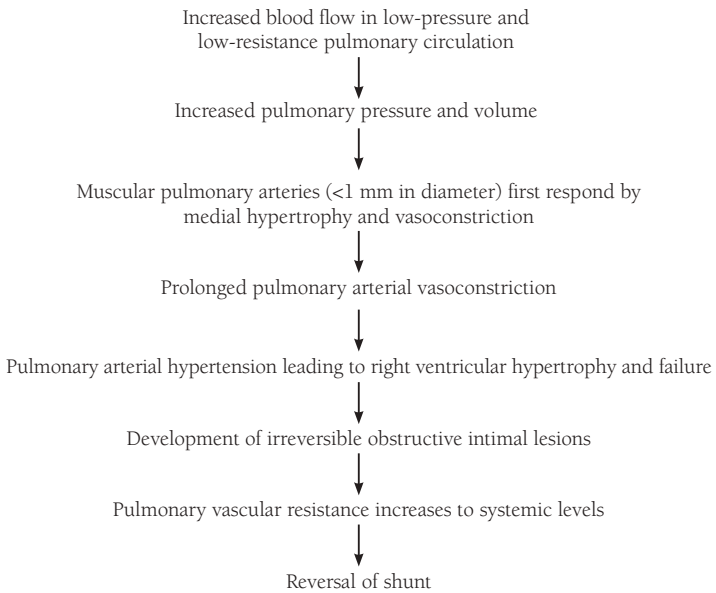
Maternal risks

Rubella, alcohol and drugs (teratogens)

Q. Classify congenital heart disease. Write briefly on the clinicopathological features of the various types of CHD.

Ans. ***Classification and clinicopathological features of various types of CHD:***

1. **Malpositions of the heart (dextrocardia)**
   (a) Apex of the heart points to the right of the chest.
   (b) May be accompanied by situs inversus; so, heart remains in normal position with respect to the other organs.
   (c) Isolated dextrocardia may be associated with major anomalies, eg, transposition of aorta and transposition of great vessels.
2. **Shunts:** Abnormal communication between chambers or blood vessels
   (a) Left to right shunt (Flowchart 11.6)



FLOWCHART 11.6. Consequences of a left to right shunt.

Reversal of shunt is also called late cyanotic congenital heart disease or **Eisenmenger syndrome.** If significant irreversible pulmonary hypertrophy develops, structural defects of congenital heart disease are considered irreparable.

Examples

- Shunts with increased pulmonary blood flow **(atrial septal defect or ASD)**
- Shunts with increased pulmonary blood flow and pressure **(ventricular septal defect or VSD** and **patent ductus arteriosus** or **PDA)**.

(i) ASD

- Abnormal opening in the atrial septum that allows communication of blood between left and right atria
- Well tolerated and asymptomatic till adulthood
- Murmur develops due to excessive blood flow through pulmonary valve
- Three major types according to the location in septum (Fig. 11.3):
  - Secundum abnormality (90% of all ASDs): Results from a deficient or fenestrated fossa ovalis
  - Primum abnormality (5% of ASDs): Occurs adjacent to atrioventricular (AV) valves; usually associated with a cleft anterior mitral (partial AV septal defect)
  - Sinus venosus defect (5% of ASDs): Located near the entrance of superior vena cava

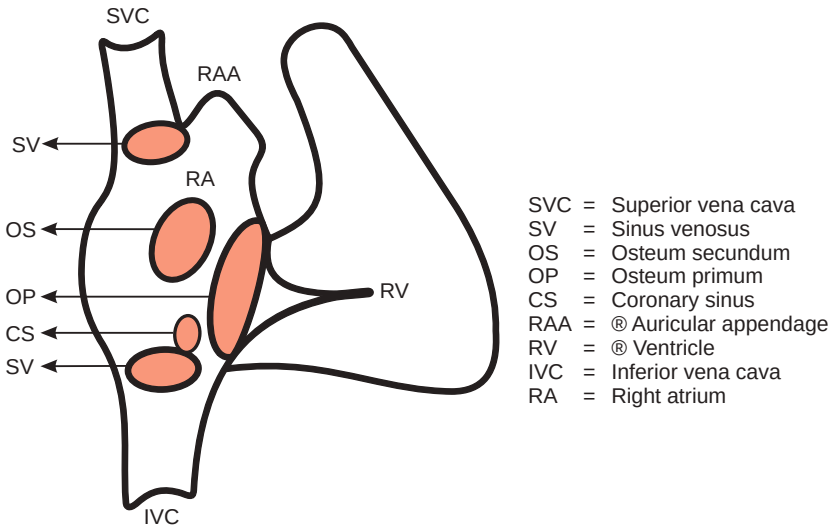


FIGURE 11.3. Schematic diagram to show various types of ASD.

Effects of ASD

- Left to right shunt at atrial level with increased pulmonary flow (because the pulmonary vascular resistance is less than the systemic vascular resistance and because the distensibility of the right ventricle is more than that of the left)
- Volume hypertrophy of right atrium and right ventricle ultimately leading to failure
- Enlargement and hemodynamic changes of tricuspid and pulmonary valves
- Volume atrophy of the left atrium and left ventricle (reduction in muscle mass) with small-sized mitral and aortic orifices
- Complications include paradoxical embolization and irreversible pulmonary disease
- ASD closure (surgical or catheter based) can completely reverse the abnormal haemodynamics

(ii) VSD

- Most common CHD (30% occur as isolated anomaly; rest associated with other defects)
- Recognized early in life
- Small defects close spontaneously while larger defects remain patent and produce significant effects

Depending upon location, VSD may be of the following types (Fig. 11.4)***:***

- 90% cases have a defect in membranous septum close to bundle of His (membranous VSD)

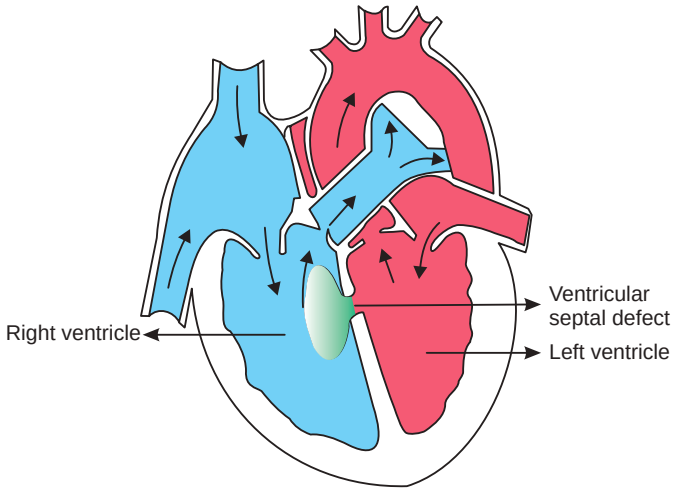


FIGURE 11.4. Schematic diagram to depict different types of VSD.

- Remaining 10% have VSD immediately below the pulmonary valve (subpulmonic) or present as multiple defects in the septum (infundibular VSD)

Effects of VSD

- Left to right shunt at the ventricular level
- Volume hypertrophy of right ventricle
- Enlargement and haemodynamic changes in the tricuspid and pulmonary valves
- Pressure hypertrophy of right atrium
- Volume hypertrophy of left atrium and ventricle
- Enlargement and haemodynamic changes in mitral and aortic valves
- Irreversible pulmonary changes

↓

Shunt reversal

↓

Cyanosis and death

(iii) PDA

- Ductus arteriosus is the vascular connection between aorta (descending) and bifurcation of pulmonary artery which normally closes within 1st to 2nd day of life.
- Persistence (due to continued synthesis of PGE2) for more than 3 months of age is abnormal and is called patent ductus arteriosus or PDA (Fig. 11.5).
- Constitutes 10% of congenital malformations of the heart and great vessels.
- Ninety percent cases present as an isolated defect; remaining are associated with VSD, coarctation and pulmonary and aortic stenosis.
- Characteristic harsh machinery-like murmur is heard on auscultation.
- A patent ductus may be up to 2 cm in length and 1 cm in diameter.
- Medical closure of PDA can be achieved with administration of indomethacin.

Effects of PDA

- Left to right shunt at the level of ductus results in increased pulmonary flow and increased volume in left heart leading to:
  - Volume hypertrophy of left atrium and ventricle

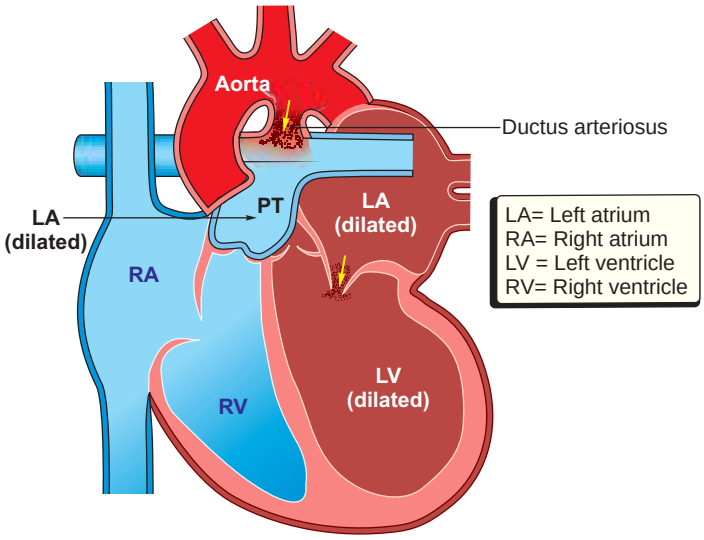


FIGURE 11.5. Schematic diagram to show PDA.

- Enlargement and haemodynamic changes of mitral and pulmonary valves
- Enlargement of ascending aorta

(iv) Patent foramen ovale

- Foramen ovale is an essential hole in the atrial septum through which oxygenated blood from the placenta travels directly from the right atrium to the left atrium without passing through the immature lungs.
- Under normal circumstances it closes at birth due to increased pressure in the left side of the heart.
- If the foramen remains patent atransient increase in the right sided pressure as is seen during coughing, sneezing or bowel movements, can produce transient right to left shunting and paradoxical embolus.

(b) **Right to left shunts**

- Blood from right side of heart enters left side.
- Dusky blueness of skin and mucous membranes (cyanosis) occurs because of poorly oxygenated blood entering systemic circulation. Also called 'cyanotic CHD'.
- 'Bland or septic emboli' arising in peripheral veins bypass pulmonary circulation (where they are normally filtered) and enter systemic circulation. These are called paradoxical emboliand may cause brain infarction and abscess.
- 'Clubbing' of tips of fingers and toes (hypertrophic osteoarthropathy) and 'polycythaemia' results from chronic longstanding cyanosis.

Examples

(i) Tetralogy of Fallot (TOF)

- Most common cyanotic CHD
- Four components (Fig. 11.6):
  - VSD usually large (shunt abnormality)
  - Displacement of aorta to the right such that, it overrides the VSD
  - Pulmonary stenosis (obstruction)
  - Right ventricular hypertrophy
- Severity of clinical features proportionate to extent of pulmonary stenosis (PS) and size of VSD

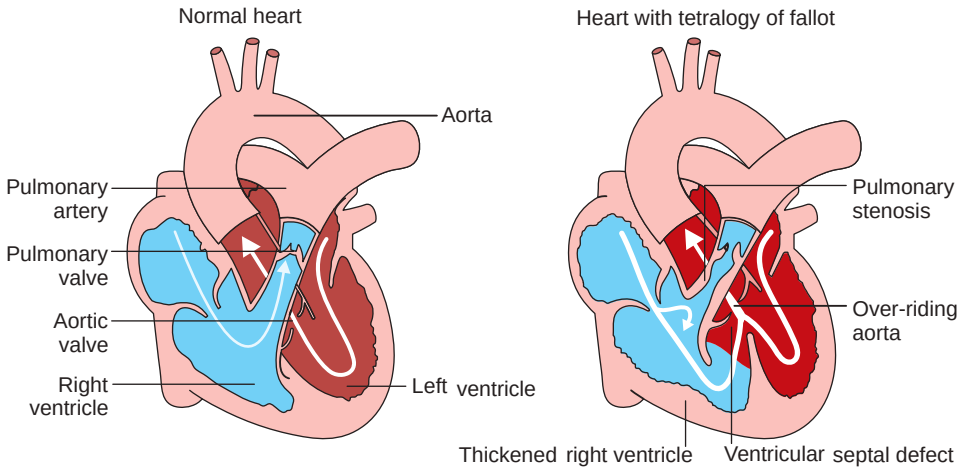


FIGURE 11.6. Schematic diagram to demonstrate TOF.

- Two types of TOF exist:
  - ***Cyanotic tetralogy*** (Flowchart 11.7)

Prominent pulmonary stenosis and mild VSD
↓
More resistance to outflow of blood from right ventricle
↓
Right to left shunt at ventricular level
↓
Cyanosis

FLOWCHART 11.7. Pathogenesis of cyanotic tetralogy.

Effects

- Pressure hypertrophy of right atrium and right ventricle
- Smaller and abnormal tricuspid valve
- Smaller left atrium and left ventricle
- Enlarged aortic orifice

Acyanotic tetralogy (Flowchart 11.8)

VSD larger, pulmonary stenosis mild
↓
Mainly left to right shunt
↓
Increased pulmonary flow
↓
Increased volume in left heart

FLOWCHART 11.8. Pathogenesis of acyanotic tetralogy.

Effects

- Pressure hypertrophy of right ventricle and right atrium
- Volume hypertrophy of left atrium and ventricle
- Enlargement of mitral and aortic orifices

- '**Boot shaped heart**' may result owing to marked right ventricular hypertrophy (particularly of apical region)
- Complete surgical repair is possible for TOF but is more complicated for patients with pulmonary atresia and dilated bronchial arteries.

(ii) Transposition of great arteries

- Complex malformations as regards position of the aorta, pulmonary trunk, atrioventricular orifices and position of atria in relation to ventricles
- Several forms of transpositions are encountered:
  - Regular transposition:
    - Most common type
    - Aorta (normally situated to the right and posterior with respect to pulmonary trunk), displaced anteriorly and to the right
    - Aorta emerges from the right ventricle and pulmonary trunk from the left ventricle so there is cyanosis from birth
  - Corrected transposition: Transposition of great arteries and veins (pulmonary veins enter the right atrium and systemic veins drain into the left atrium) resulting in 'physiological correction'

(iii) Persistent truncus arteriosus:

- Arch separating aorta from pulmonary artery fails to develop.
- Single large common vessel receives blood from right and left ventricles.
- Associated VSD is common.
- Left to right shunt and early systemic cyanosis is typical.

(iv) Tricuspid atresia and stenosis:

- Rare; often associated with pulmonary stenosis or atresia
- May be accompanied by an interatrial defect through which right to left shunting of blood takes place leading to early cyanosis

3. **Obstructive CHD**

(a) Coarctation of aorta

- Localized narrowing in any part of aorta commonly located below the origin of the subclavian artery
- May be associated with a bicuspid aortic valve in 70% cases, Berry aneurysm in the circle of Willis and Turner syndrome
- Two main types:
  - Postductal or adult type:
    - Obstruction distal to the point of entry of ductus arteriosus
    - Aorta is dilated on either side of the constriction
    - Condition usually recognized in adulthood when it manifests with hypertension in upper extremities, weak pulses and low blood pressure in the lower extremities
    - Effects of arterial insufficiency, eg, claudication and coldness are common
    - Collateral circulation between prestenotic and poststenotic arterial branches leads to enlarged and palpable intercostal arteries
    - Erosions on the inner surface of ribs may be seen
  - Preductal or infantile type:
    - Narrowing is proximal to ductus arteriosus (aortic arch, ascending aorta)
    - Manifests with cyanosis in lower half of the body; upper half remains unaffected as it is supported by vessels originating proximal to the coarctation
    - Has a poor prognosis

(b) Aortic stenosis and atresia

- Most common anomaly of aorta is a bicuspid aortic valve which has no functional significance except calcification.
- Congenital aortic atresia is rare and incompatible with survival.
- Aortic stenosis may be congenital or acquired. Causes of the latter include RHD and calcified aortic stenosis.
- Congenital narrowing of the aortic valve can occur in the valvular, subvalvular and supravalvular locations.

(c) **Pulmonary stenosis and atresia**
- Pulmonary atresia: No communication between right ventricle and lungs. Blood bypasses the right ventricle through interatrial defect.
- Pulmonary stenosis: Commonest form of obstructive CHD; comprising about 7% of all CHDs; may be an isolated defect or part of TOF.

Q. Write in detail on the aetiopathogenesis, clinical features and laboratory diagnosis of acute rheumatic fever and rheumatic heart disease.

Ans. Acute rheumatic fever is an acute immune-mediated multisystem disease, which primarily involves the heart, joints, central nervous system, skin and subcutaneous tissues.

- Its peak incidence is between 5 and 15 years and it is rare in infants and children below the age of 5 years.
- It is more common in poor economic conditions and overcrowding.
- ***It progresses over course of time to chronic rheumatic heart disease.***

Aetiology

- It is a delayed inflammatory response to pharyngeal infection with group A streptococci.
- The latent period between the pharyngeal infection and the onset of rheumatic fever ranges from 1 to 5 weeks.
- Type 5 strain commonly causes rheumatic fever. The other rheumatogenic serotypes include 1, 3, 6, 14, 18, 19 and 24.
- Antibodies develop against streptococcal antigens but cross react with cardiac myosin and sarcolemmal membrane protein.

Pathology

Acute rheumatic fever is characterized by exudative inflammatory lesions of connective tissue mainly involving the heart, joints and subcutaneous tissues. These exudative lesions are replaced by scar tissue in the later or healed phase of the disease (chronic rheumatic heart disease). All three layers (endocardium, myocardium and pericardium) of the heart are involved resulting in **pancarditis**. The following pathological changes are seen in the different layers:

- **Myocardium:** Myocardium shows the pathognomonic myocardial **Aschoff body** (focus of swollen eosinophilic collagen surrounded by lymphocytes, plasma cells, fibroblasts, Aschoff giant cells and large basophilic cells called **Anitschkow cells**, which have abundant cytoplasm, round to oval nuclei with central slender wavy ribbon like chromatin; therefore, Anitschkow cells are also called caterpillar cells). Aschoff bodies are classically located in the interstitial connective tissue of myocardium especially in perivascular location and may persist for many years in chronic rheumatic inflammation, especially in those who develop severe mitral stenosis.
- **Endocardium:** Rheumatic endocarditis produces verrucous lesions, which heal with fibrous thickening and adhesions of valve commissures, leaflets and chordae tendinae, resulting in varying degrees of stenosis and regurgitation. Regurgitant streams produce irregular thickening of the left atrium called **MacCallum plaques.** Mitral valve is the most commonly involved, followed by aortic valve, and rarely, tricuspid valve. Pulmonary valve is almost never involved.
- **Pericardium:** Serofibrinous pericarditis produces a classical '**bread and butter**' appearance.

Clinical Manifestations

1. **Sore throat:** History of antecedent upper respiratory tract infection in the past 1–5 weeks.

2. **Polyarthritis**
   (a) Arthritis is the most common major manifestation of rheumatic fever (present in nearly 75% of cases).
   (b) Classical presentation is acute migratory or fleeting polyarthritis (most commonly of large joints of the extremities). As pain and swelling subside in one joint, others tend to get involved.
   (c) Involvement of hips, small joints of hands and feet, spine, sternoclavicular and temporomandibular joints is rare.
   (d) Affected joints show signs of inflammation with or without effusion.
   (e) Over a period of time, involved joints heal without any residual deformity.
3. **Carditis**
   (a) Features of carditis develop early (within 3 weeks of onset) and occur in 40–50% of cases.
   (b) May be asymptomatic and picked up on echocardiography only.
   Manifestations of carditis
   - Myocarditis
     - Tachycardia, disproportionate to fever and persisting during sleep
     - Arrhythmias of which prolongation of PR interval is the most common
     - Features of congestive heart failure and cardiomegaly
   - Endocarditis
     - Apical systolic murmur of mitral regurgitation
     - Apical mid-diastolic murmur (Carey Coombs murmur) due to nodules on the mitral valve leaflets
     - Basal early diastolic murmur of aortic regurgitation
   - Pericarditis
     - Pericardial pain
     - Pericardial friction rub
     - Pericardial effusion (uncommon and always small)

   Diagnosis of carditis requires the presence of one or more of the following:
   - Appearance of, or change in the character of organic murmurs
   - Cardiomegaly
   - Pericarditis or pericardial effusion
   - Congestive heart failure
4. **Subcutaneous nodules**
   (a) Associated with severe carditis and tend to occur several weeks after its onset (delayed manifestation)
   (b) Manifest as small, painless nodules over extensor surfaces and bony prominences
5. **Erythema marginatum**
   (a) Occurs in nearly 10% cases of acute rheumatic fever
   (b) Characterized by erythematous macules with a clear centre and serpiginous margins, most commonly seen on the trunk and proximal parts of extremities
6. **Chorea (Sydenham chorea; chorea minor; Saint Vitus Dance)**
   (a) Chorea usually appears following a long latent period (up to 6 months) after the initial streptococcal infection
   (b) Purposeless, involuntary movements associated with muscle weakness, emotional instability, tics and psychotic features characterize chorea

Chronic Rheumatic Fever

The average duration of an untreated attack of acute rheumatic fever is approximately 3 months. Chronic rheumatic fever is defined as disease persisting for longer than 6 months and may be a cause of persisting congestive heart failure.

Laboratory investigations for Acute Rheumatic Fever:

1. **Isolation of group A streptococci:** Positive throat swab cultures for group A streptococci
2. **Streptococcal antibody tests (serologic tests):**
   (a) These tests confirm a recent streptococcal infection.

(b) Common serologic tests done are
  (i) Antistreptolysin O (ASO) levels: Single titer of ASO more than 200 Todd units in adults and 300 Todd units in children above 5 years, are taken as positive. However, a rising titer is even more significant.
  (ii) Anti-DNase B levels
  (iii) Antihyaluronidase (AH) levels
  (iv) Antistreptozyme test (ASTZ) levels: Very sensitive indicator of recent streptococcal infection. Titers more than 200 U/mL are considered positive

3. **Acute phase reactants:** These tests confirm the presence of an inflammatory process, but are nonspecific:
   (a) Raised erythrocyte sedimentation rate (ESR)
   (b) Increased C-reactive protein (CRP)
4. **Haematologic abnormalities and levels of serum proteins:**
   (a) Neutrophilic leukocytosis
   (b) Increase in serum complement level
   (c) Increase in serum mucoproteins, alpha-2 and gamma-globulin levels
   (d) Anaemia due to suppression of erythropoiesis
5. **Electrocardiogram:** The most consistent abnormality is prolongation of the PR interval.
6. **X-ray chest:** Evidence of cardiac failure may be seen, like:
   (a) Cardiomegaly
   (b) Pulmonary congestion
7. **Echocardiography:** Echocardiography may show
   (a) Myocardial dysfunction
   (b) Valvular dysfunction
   (c) Pericardial effusion

Jones criteria for diagnosis of acute rheumatic fever are summarized in Table 11.4**.**

TABLE 11.4. Diagnosis of acute rheumatic fever (Jones criteria)

Major manifestations	Minor manifestations
Carditis	Fever
Polyarthritis	Arthralgia
Chorea	Previous rheumatic fever or rheumatic heart disease
Erythema marginatum	Raised ESR
Subcutaneous nodules	Positive CRP
	Prolonged PR interval (first-degree A–V block)

Supporting evidence of preceding streptococcal infection includes:

1. Recent scarlet fever
2. Positive throat culture for group A streptococcus
3. Increased streptococcal antibodies

Two major manifestations or one major and two minor manifestations, along with at least one supporting evidence, indicate a high probability of rheumatic fever.

Q. Classify endocarditis. Write briefly on the clinicopathological features of the various types of endocarditis.

Ans. Endocarditis is the inflammatory involvement of the endocardial layer of heart including valves, chordae and papillae.

Classification

1. **Noninfective**
   (a) Rheumatic endocarditis (see answer to previous question)
   (b) Atypical verrucous (Libman–Sacks) endocarditis
   (c) Nonbacterial thrombotic (NBTE) or marantic endocarditis

2. **Infective**
   (a) Bacterial endocarditis
   (b) Other infective types (tuberculous, syphilitic and fungal)

Atypical Verrucous (Libman–Sacks) Endocarditis

Pathogenesis

This type of endocarditis is characterized by formation of sterile vegetations in patients of collagen vascular diseases (SLE, systemic sclerosis and thrombotic thrombocytopenic purpura). These lesions are essentially composed of fibrinoid material thought to result from activation of complement system and recruitment of Fc receptor bearing cells.

Gross Pathology

- Small (1–4 mm in diameter), single or multiple, granular-pink vegetations typically seen in the mitral and tricuspid valves
- Both surfaces of affected valves, adjoining ventricular and atrial endocardium are involved.
- Healed lesions do not produce any significant valvular deformity.

Microscopy

- Verrucae composed of fibrinoid material (fibrin + platelet thrombi) are pathognomonic.
- Valves show areas of necrosis, proliferating capillaries, histiocytes, plasma cells, lymphocytes and neutrophils.

Nonbacterial Thrombotic/Cachectic/Marantic Endocarditis

Pathogenesis

Not clear; typically show presence of sterile thrombotic vegetations in association with:

(a) Chronic debilitating diseases, eg, cancers, particularly, mucin-secreting adenocarcinomas (which show increased blood coagulability or DIC due to the procoagulant effect of mucin) and nonmucin-secreting malignancies, eg, promyelocytic leukaemia (promyelocyte granules contain procoagulant substances)
(b) Hyperestrogenic states, severe burns or sepsis, which promote hypercoagulability
(c) Endocardial trauma due to an indwelling catheter

Gross Pathology

- Small (1–5 mm in diameter) single or multiple vegetations seen along the line of closure of the leaflets
- More friable than vegetations of RHD
- Healed vegetations form fibrous nodules

Microscopy

Bland thrombi (platelets and inflammation) without accompanying inflammatory reaction or bacteria

Complications

Embolization may result in infarcts in brain, lung, spleen and kidney.

Bacterial Endocarditis

Caused by invasion of heart valves or mural endocardium by a microbe. May be classified into acute or subacute types depending on the virulence of the organism and cardiac status of the patient.

(a) Acute bacterial endocarditis
   (i) Destructive, necrotizing and ulcerative invasion by highly virulent bacteria
   (ii) Usually affects normal valves
   (iii) Death within days to weeks in 50% patients, despite antibiotics and surgery

(b) Subacute bacterial endocarditis
  (i) Caused by less destructive and less virulent microorganisms
  (ii) Usually affects previously diseased heart

Predisposing Factors

- Conditions which initiate transient bacteraemia and septicaemia
  - Periodontal infections
  - Genitourinary tract infections
  - Infections of gastrointestinal and urinary tract
  - Tonsillectomy, adenoidectomy, bronchoscopy and surgery on respiratory mucosa
- Underlying heart disease
  - RHD
  - Congenital heart disease (VSD, subaortic stenosis, myxomatous mitral value and artificial valves)
- Impaired host defences
  - Immunodeficiency
  - Diabetes mellitus
  - Neutropenia or deficient function of neutrophils

Causative Agents

- *Staphylococcus aureus* (in 10–20% cases); affects healthy or diseased heart and is the major offender in intravenous drug users
- *Streptococcus viridians* (in 50–60% cases); causes endocarditis in previously damaged/abnormal valves
- *Staphylococcus epidermidis* (in 10% cases) is the most common cause of prosthetic valve endocarditis
- Other organisms implicated are the **HACEK group** (*Hemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella* and *Kingellakingae*)

Gross Pathology

- Valves of left heart, ie, mitral and aortic are most frequently affected
- Vegetations involve atrial surface of AV valves and ventricular surface of semilunar valves
- Size varies between a few mm to cm; the vegetations are grey, single or multiple and friable
- Acute bacterial endocarditis vegetations are bulkier than subacute endocarditis

Microscopy

The vegetations have three recognizable zones:

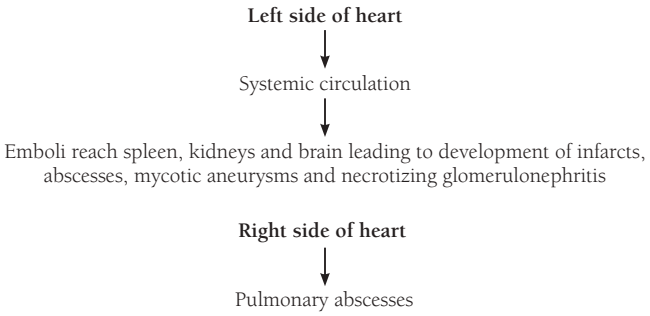
- Outer layer or cap composed of eosinophilic material (fibrin and platelets)
- Basophilic zone composed of bacterial colonies
- Deeper zone composed of nonspecific inflammatory cells

Clinical Features

Fever with chills, weakness, malaise and weight loss

Complications and Sequelae

- Cardiac complications:
  - Valvular stenosis or insufficiency
  - Perforation, rupture, aneurysm formation
  - Abscess in valve ring extending to myocardium
  - Suppurative pericarditis
  - Cardiac failure
- Extracardiac complications:
  - Emboli can arise from the left or right side of the heart



- Microembolization may result in
  - **Petechiae** (on the skin and conjunctiva due to involvement of local vessels)
  - **Osler's nodes** (painful tender nodules seen in pulps of fingers, thought to form due to deposition of immune complexes)
  - **Roth's spots** (involvement of retinal vessels may result in circular retinal haemorrhages with pale centres)
  - **Jane way spots** on palms and soles (due to septic emboli in the skin)
  - **Subungual splinter haemorrhages** (due to embolic damage to cutaneous capillaries)
  - **Painful splenomegaly** (due to splenic vessel involvement)

Causes of Death

- Cardiac failure
- Embolism to various organs
- Renal failure
- Rupture of mycotic aneurysms in vital organs

Rarely, infectious endocarditis can also result from tuberculous, syphilitic, fungal, viral and rickettsial infection.

Laboratory Studies

- Normocytic normochromic anaemia of chronic disease with leucocytosis
- Increased ESR
- Increased levels of CRP (C-reactive protein)
- Two sets of positive blood cultures including aerobic, anaerobic and fungal (90% sensitivity) of three specimens taken at intervals of 2–3 h
- Deranged coagulation panel
- Increased BUN and serum creatinine
- Decreased C3 and C4 levels
- Proteinuria and microscopic haematuria in 50% patients
- Echocardiographic detection of vegetations, valve lesion and chamber dilatation

Diagnostic Criteria for Infective Endocarditis (Modified Duke's Criteria)

1. Major criteria
   (a) Positive blood culture for infective endocarditis (IE)
      (i) Typical microorganism of IE from two separate blood cultures (*Streptococcus viridans, Streptococcus bovis*, or HACEK group, community acquired *Staphylococcus aureus* or enterococci, in the absence of a primary focus), or
      (ii) Microorganisms consistent with IE from persistently positive blood cultures defined as:
         - Two positive cultures of blood samples drawn >12 h apart, or
         - All of three or a majority of four separate cultures of blood (with first and last sample drawn 1 h apart).
   (b) Evidence of endocardial involvement
      (i) Positive echocardiogram for IE, or
      (ii) Abscess or new partial dehiscence of prosthetic valve, or
      (iii) New valvular regurgitation (worsening or changing of preexisting murmur not sufficient)
2. Minor criteria
   (a) Predisposing valvular lesion

(b) Fever
(c) Embolic phenomenon
(d) Vasculitic phenomenon
(e) Microbiologic evidence (organism grown but does not meet major criteria)
(f) Suggestive echocardiographic findings

Definite endocarditis: Two major or one major and three minor or five minor criteria
Possible endocarditis: One major and one minor or three minor criteria

Q. Enumerate the differences between acute and subacute endocarditis.

Ans. The differentiating features of acute and subacute endocarditis are enlisted in Table 11.5.

TABLE 11.5. Differentiating features of acute and subacute endocarditis

Features	Acute bacterial endocarditis	Subacute bacterial endocarditis
Duration	<6 weeks	>6 weeks
Causative organisms	*Staphylococcus aureus*, β-streptococci	*Streptococcus viridans*
Virulence of organisms	High	Low
Preexisting pathology	None	Previously damaged valves
Lesion characteristics	Large invasive and destructive	Less invasive and destructive
Clinical presentation	Rapid deterioration, usually fatal	Gradual downhill course or recovery

Note: Classification of infective endocarditis into acute and subacute forms is largely discarded due to availability of antibiotics, which alter the course of the same.

Q. Compare the morphological features of different types of endocarditis.

Ans. The distinguishing features of vegetations in major forms of endocarditis are summarized in Table 11.6.

TABLE 11.6. Distinguishing features of vegetations in major forms of endocarditis (Fig. 11.7)

Features	Rheumatic	Libman sacks	Bacterial	NBTE
Valves commonly affected	Mitral alone or mitral and aortic combined	Mitral and tricuspid	Mitral, aortic, or combined mitral and aortic	Mainly mitral; less commonly aortic and tricuspid
Distribution of vegetations	Occur along lines of closure; atrial surface of A–V valves and ventricular surface of semilunar valves	Occur on cusps/leaflets either on one or both surfaces	On valve cusps, may extend into the chordate	Occur along lines of closure (on one side)
Gross appearance	Small (1–5 mm), multiple, warty, firmly attached, produce thickening, and shortening of leaflets as well as fusion of valve commissures leading to permanent valvular deformity	Small to medium sized, multiple vegetations; generally do not produce much deformity	Large irregular, destructive, friable masses, damage the underlying myocardium	Small but larger than RHD; loosely attached; usually do not damage the valve
Constituents	Fibrin and platelets, no bacteria; adjacent endocardium may show presence of **Aschoff bodies**	Granular, fibrinoid, eosinophilic material; sterile vegetations; may contain **haematoxylin bodies** equivalent to LE cells	Fibrin and platelets with bacterial colonies and acute inflammatory cells	Fibrin–platelet thrombi, no bacteria
Embolization	Less common	Less common	Friable vegetations; very common	Very common

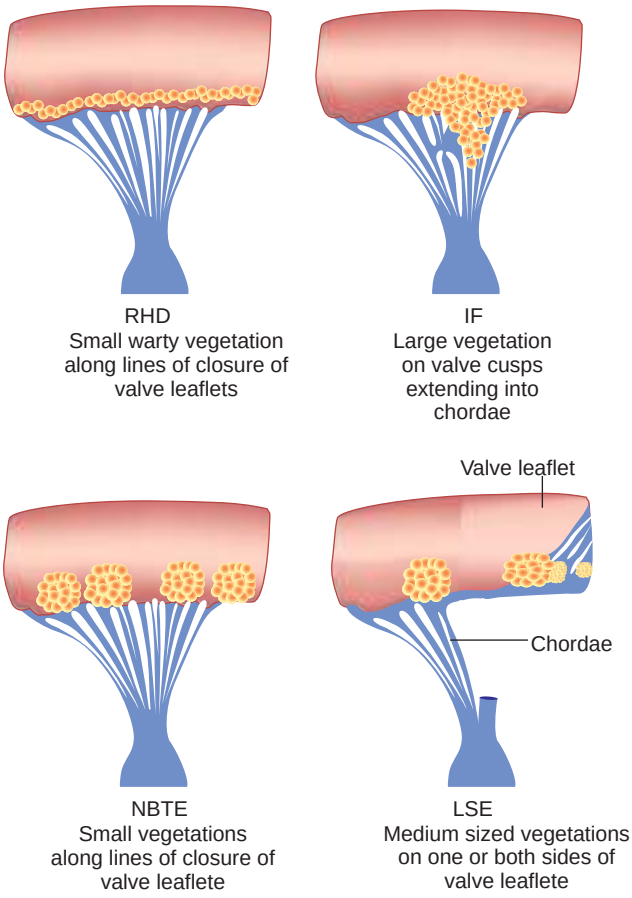


FIGURE 11.7. Types of endocardial vegetations.

Q. Write briefly on the aetiology and clinicopathological features of myocarditis.

Ans. Myocarditis is inflammatory involvement of the myocardium, which has diverse causes. It is classified based on aetiology into

1. **Infective myocarditis**
   - (a) Viral (*coxsackie, ECHO, influenza, HIV*)
   - (b) Chlamydial (*Chlamydophyla psittachi*)
   - (c) Rickettsial (*Rickettsia typhi*)
   - (d) Bacterial (*C. diphtheriae*, *Neisseria meningococcus*, *Borrelia*)
   - (e) Fungal (*Candida*)
   - (f) Helminthic (*Trichinosis*)
   - (g) Protozoal (*Trypanosoma*, *Toxoplasma*)
2. **Immunological myocarditis**
   - (a) Connective tissue diseases (rheumatoid arthritis, lupus erythematosus, polyarteritis nodosa and scleroderma)
   - (b) Drug hypersensitivity (methyldopa and sulphonamides)
   - (c) Postviral
   - (d) Poststreptococcal
   - (e) Transplant rejection
3. **Myocarditis of unknown origin**
   - (a) Sarcoidosis
   - (b) Giant cell myocarditis

Infective myocarditis

This is the most common type of myocarditis. It results from direct invasion of causative organisms or their toxins into the myocardium or a destructive immune response to their antigens. Common causes include

- **Viral**
  - Seen in 5% of viral infections due to direct viral cytotoxicity or a destructive cell-mediated immune reaction
  - Myocardium is pale and flabby; focal or patchy necrosis and mural thrombi may be seen on gross examination. Microscopy shows oedema and infiltration by neutrophils, which in the later stages are replaced by lymphocytes and macrophages
- **Bacterial**
  - Myocardium shows abscesses
  - Exudate is chiefly composed of neutrophils, lymphocytes, plasma cells and macrophages
  - Coagulative necrosis may be seen
- **Helminthic**
  Echinococcus granulosus and *Trichinella spiralis* are generally implicated.
- **Fungal**
  Seen in immune deficiency, cancer and chronic debilitating diseases
- **Protozoal (Chagas disease)**
  Mixed inflammation around parasitized myofibres is seen.

Q. Enumerate and describe the various types of cardiomyopathies.

Ans. Cardiomyopathy (CMP) is a heterogeneous group of diseases associated with mechanical and electrical dysfunction of the myocardium. Cardiomyopathies may be localized to the heart or may form a part of a systemic disease.

1. **Primary cardiomyopathy:** Diseases confined to heart muscle. May be genetic or acquired in origin.
   Three pathophysiologic categories are encountered (Table 11.7):
   (a) Dilated (congestive) CMP
   (b) Hypertrophic (obstructive) CMP
   (c) Restrictive/obliterative or infiltrative CMP
   Further, restrictive cardiomyopathy includes the following entities:
   (i) Cardiac amyloidosis
   (ii) Endocardial fibroelastosis
   (iii) Endomyocardial fibrosis
   (iv) Loeffler endocarditis
2. **Secondary cardiomyopathy:** The myocardial involvement is seen as a component of a systemic or a multiorgan disorder, as in
   (a) Nutritional deficiencies (alcoholic CMP and beriberi)
   (b) Toxic chemicals (cobalt, arsenic, lithium and hydrocarbons)

TABLE 11.7. Types of CMP

Features	Dilated (congestive) CMP	Hypertrophic (obstructive) CMP	Restrictive/obliterative or infiltrative CMP
Definition	• Systolic dysfunction with dilatation of all four chambers	• Diastolic dysfunction (reduced chamber size and impaired diastolic filling) • 25% patients have left ventricular outflow obstruction	• Diastolic dysfunction (restriction in ventricular filling due to reduction in the volume of ventricles)
Incidence	90% of all CMPs	Less common	Less common
Age affected	20–50 years	25–40 years	Variable

TABLE 11.7. Types of CMP—cont'd

Features	Dilated (congestive) CMP	Hypertrophic (obstructive) CMP	Restrictive/obliterative or infiltrative CMP

Causes	• **Familial or genetic:**
	  • Inheritance is mainly autosomal dominant
	  • X-linked, autosomal recessive or mitochondrial inheritance less common
	  • Inherited mutations seen in proteins like dystrophin, cardiac α actin, desmin and nuclear proteins like lamin A and C
	• **Acquired:**
	  • Viral myocarditis (Enterovirus and coxsackie virus implicated)
	  • Toxic damage from alcohol (direct toxicity or beriberi heart disease due to thiamine deficiency), cobalt, chemotherapy with doxorubicin and other anthracyclines
	  • Peripartum CMP
	  • Iron overload as in hereditary haemochromatosis
	  • Excessive catecholamines as in pheochromocytoma
	  • Supraphysiologic or extreme psychological stress
		• **Genetic; inherited mutations in sarcomere proteins** (β-myosin heavy chain or troponin I and T, myosin binding protein C and α-tropomyosin)	Idiopathic; or associated with amyloidosis, radiation induced fibrosis, sarcoidosis, metastatic tumour or inborn errors of metabolism

Clinical features	Slowly progressing heart failure, shortness of breath, easy fatigability, poor exercise tolerance	Usually asymptomatic; symptomatic on heavy physical activity. May manifest with dyspnoea, angina and congestive cardiac failure or sudden death	Presentation dependent on specific type. May manifest with dyspnoea, angina and congestive cardiac failure or sudden death
Gross appearance	• Heart is enlarged, weight may increase up to 1000 g ('**flabby hypocontracting heart**'). • Dilatation of all four chambers giving rise to a typical globular appearance. • Endocardial thickening • Presence of mural thrombi	• Heavy hypercontracting heart • Asymmetric myocardial hypertrophy (interventricular septum more hypertrophied than free walls of the ventricles) • Transverse section: banana-like appearance	• Ventricles normal or slightly enlarged • Cavities not dilated • Myocardium is firm
Microscopy	• Hypertrophy of some myocardial fibres; atrophy of others. Interstitial fibrosis with focal mononuclear infiltrate. • Small subendocardial scars may be seen	• Myocardial cell disorganization in the ventricular septum • Myocardial fibres are irregularly and haphazardly arranged (normally parallel) • Interstitial fibrosis • Individual muscle hypertrophy and presence of large prominent nucleoli (transverse diameter more than 40 cm)	Patchy or diffuse interstitial fibrosis, which varies from minimal to extensive
Outcome	Mitral regurgitation/arrhythmias may be observed. Average survival from onset to death is 5 years	Medical treatment to relax ventricles and surgical reduction of septum can be undertaken	Gradually progressive cardiac failure

(c) Drugs (emetrine, cyclophosphamide, adriamycin and catecholamines)
(d) Metabolic diseases (cardiac amyloidosis, hemochromatosis and glycogen storage diseases)
(e) Neuromuscular diseases (Friedreich ataxia and muscular dystrophies)
(f) Infiltration by leukaemias and carcinomas
(g) Connective tissue diseases (rheumatoid arthritis, systemic sclerosis and drug induced)

Q. Write briefly on diseases of the pericardium.

Ans. Pericardium

- The visceral pericardium is a simple layer of mesothelial cells and the parietal pericardium is made of fibrous and elastic tissue. It is usually about 2 mm thick and well innervated.
- Diseases of the pericardium are usually secondary to or associated with other cardiac and systemic diseases.
- Normally, no more than 30–50 mL of thin, clear, straw-coloured fluid is seen in pericardial sac.
- Pericardial cavity may undergo distension by fluid (effusion), blood (haemopericardium) or pus (purulent pericarditis). When significant fluid accumulates in the pericardial cavity, it can cause *cardiac tamponade,* which is characterized by rapidly declining cardiac output progressing to cardiogenic shock.
- Pericarditis (inflammation of the pericardium) may be classified based on the onset (acute or chronic) and aetiology (serous, fibrinous, purulent, haemorrhagic and chylous).

Classification of Pericarditis

1. **Classification of pericarditis based on onset:**
   a) Acute
   b) Chronic
2. **Classification of pericarditis based on aetiology:**
   a) Acute nonspecific (idiopathic)
   b) Infective (bacterial, viral, tubercular, fungal or other infections)
   c) Immunologic (rheumatic fever or other connective tissue disorders)
   d) Neoplastic
   e) Metabolic (uraemia, myxoedema or gout)
   f) Traumatic (including post–cardiac surgery)

Acute Pericarditis

1. **Serous pericarditis**
   Accumulation of serous fluid which differs from transudate of hydropericardium in having increased protein content and higher specific gravity.

Causes:

(a) Viral infection, eg, mumps, coxsackie A or B
(b) Rheumatic fever
(c) Rheumatoid arthritis, Systemic Lupus Erythematosus (SLE) and scleroderma
(e) Uraemia
(f) Involvement by a malignant tumour
(g) Tuberculous pericarditis in early stage

Pathology:

- Usually the volume of the fluid does not exceed 50–200 mL.
- There is infiltration of epicardial and pericardial surfaces by scant neutrophils, lymphocytes and histiocytes.
- Clear, watery fluid; specific gravity < 1.015

2. **Fibrinous and serofibrinous pericarditis**
   Fibrinous (bread and butter) pericarditis is the most common type of pericarditis. It may resolve or lead to adhesive pericarditis. A pericardial frictional rub can be elicited when it is active.

Causes:

(a) Acute viral illness
(b) Uraemia
(c) Myocardial infarction (postinfarction Dressler syndrome)
(d) Rheumatic fever
(e) Trauma
(f) Acute bacterial infections

Pathology:

- Pericardial surface is dry, granular
- Pericardial fluid appears thick yellow or cloudy because of abundant leukocytes and erythrocytes
- During course of evolution fibrin may get digested or become organized

3. **Purulent pericarditis**

It is a consequence of invasion of the pericardial space by infective organisms, which reach by the following routes:

a) Direct extension from neighbouring inflammation, eg, empyema, lobar pneumonia, mediastinal infections, etc.
b) Seeding from blood
c) Lymphatic extension
d) Direct introduction during cardiotomy

Most common causative organisms are Staphylococci, Streptococci and Pneumococci. The patient presents with high grade fever, rigors and a pericardial friction rub.

Pathology:

- Serosa reddened, granular and coated with exudate (thin creamy pus)
- Organization of exudate may lead to **constrictive pericarditis**

4. **Haemorrhagic pericarditis**

It is characterized by an exudate composed of blood mixed with fibrinous or suppurative material. Its causes include

a) Malignant neoplastic involvement of the pericardium
b) Bacterial infections
c) Bleeding diathesis
d) Tuberculosis
e) Following cardiac surgery

5. **Chylous pericarditis**

This type of pericarditis occurs due to lymphatic obstruction.

Chronic Pericarditis

1. **Tubercular/caseous pericarditis:** Pericardial involvement occurs by direct spread from a tuberculous focus in tracheobronchial lymph nodes and usually results in disabling constrictive pericarditis.
2. **Chronic adhesive pericarditis:** Fibrinous, suppurative or haemorrhagic pericarditis heal by organization and lead to formation of fibrous adhesions between parietal and visceral pericardium. This is usually seen due to infection by pyogenic bacteria and tuberculosis.
3. **Chronic constrictive pericarditis:** Rarely the pericardial space may get filled with fibrous or fibrocalcific tissue which interferes with normal functioning of the heart. It is usually a consequence of tuberculous, purulent or haemorrhagic pericarditis.

Q. Define heart failure. Discuss the types, common causes, pathophysiology and clinical features of heart failure.

Ans. Heart failure is defined as a state in which the ventricles cannot maintain an adequate cardiac output to meet the metabolic needs of the body.

Pathophysiology

- **Cardiac output** is a function of
  - **Preload,** which refers to the volume and pressure of blood in the ventricles at the end of diastole

- **After load**, which refers to the pressure against which the left ventricle contracts. The main determinants of after load are total peripheral resistance and left ventricle size
- **Myocardial contractility** mainly depends on the levels of circulating catecholamines

- Heart failure is characterized by a decrease in cardiac output (except in high output failure) increased preload, as well as after load and decreased myocardial contractility.
- **Compensated heart failure:** Compensated heart failure implies that the compensatory changes have prevented the development of overt heart failure; a minor added insult like an infection may precipitate severe heart failure.
- **Compensatory mechanisms** are mediated through renin–angiotensin system and autonomic nervous system and include
  - Increased myocardial contractility
  - Myocardial hypertrophy
  - Neurohormonal mechanisms:
    (i) Sympathetic stimulation
    (ii) Activation of renin–angiotensin system
    (iii) Release of atrial natriuretic peptide

Types of heart failure

- Systolic and diastolic heart failure
  - Systolic heart failure is characterized by an abnormality of myocardial contraction.
  - Diastolic heart failure is characterized by an abnormality of ventricular relaxation, which causes poor ventricular filling and high filling pressure.
- Acute and chronic heart failure
  - Acute heart failure develops suddenly. The sudden reduction in cardiac output results in systemic hypotension without peripheral oedema, eg, acute myocardial infarction and rupture of a cardiac valve.
  - Chronic heart failure develops gradually. Here, systemic arterial pressure is well-maintained, but oedema develops, eg, dilated cardiomyopathy and multivalvular disease.

- **Left-sided, right-sided and biventricular heart failure**
  - Left-sided (left ventricular) heart failure
    - 'Left side' is a term for the functional unit of left atrium, left ventricle, mitral valve and aortic valve.
    - There is reduction in left ventricular output, increase in left atrial pressure and increase in pulmonary venous pressure.
    - Acute increase in left atrial pressure causes pulmonary congestion and pulmonary oedema, eg, in myocardial infarction.
    - Gradual increase in left atrial pressure causes reflex pulmonary hypertension, but no pulmonary oedema, eg, aortic stenosis.
  - Right-sided (right ventricular) heart failure
    - 'Right side' is a term for the functional unit of right atrium, right ventricle, tricuspid valve and pulmonary valve.
    - There is reduction in right ventricular output, which results in systemic venous congestion leading to accumulation of fluid in the body, resulting in swelling and oedema.
    - Seen in corpulmonale, pulmonary valvular stenosis and multiple pulmonary emboli.
  - Biventricular heart failure: There is failure of both left and right ventricles, eg, disease processes affecting both ventricles like dilated cardiomyopathy and ischaemic heart disease or disease of left heart leading to chronic elevation of left atrial pressure, pulmonary hypertension and subsequent right ventricular failure.
- **Forward and backward heart failure**
  - *Forward heart failure:* decreased cardiac output
  - *Backward heart failure:* normal cardiac output, but marked salt and water retention and pulmonary and systemic venous congestion
- **High output and low output heart failure**
  - High output heart failure is associated with an increased cardiac output, eg, cardiac failure associated with hyperthyroidism, anaemia, pregnancy, arteriovenous fistulae, beriberi and Paget disease.

- Low output heart failure is associated with a low cardiac output, eg, heart failure associated with ischaemic heart disease, hypertension, cardiomyopathy, valvular diseases and pericardial disease.

Common Causes of Heart Failure

- ***Failure of myocardial pump***
  - Myocardial infarction
  - Hypertensive heart disease
  - Myocarditis
  - Cardiomyopathies
  - Pulmonary embolism
  - Anaemia, pregnancy and thyrotoxicosis (increased demand)
  - Drugs like beta-blockers, corticosteroids and NSAIDs
- ***Obstruction to outflow***
  - Systemic hypertension
  - Aortic and pulmonary stenosis
  - Pulmonary hypertension
- ***Obstruction to inflow:*** Mitral and tricuspid stenosis
- ***Disorders of cardiac conduction:*** Cardiac arrhythmias

Clinical Manifestations of Heart Failure

- ***Dyspnoea:*** Dyspnoea is initially exertional, but progressively worsens to a stage of breathlessness even at rest. The mechanisms underlying dyspnoea include
  - Interstitial pulmonary oedema
  - Respiratory muscle dysfunction/fatigue due to poor perfusion
  - Bronchial mucosal oedema and increased bronchial mucous production
- ***Orthopnoea:*** Dyspnoea occurring in recumbent position (on lying flat) and relieved by sitting up is known as orthopnoea. The mechanisms underlying orthopnoea include
  - Increased venous return to the lungs in recumbent position
  - Elevation of the diaphragm
  - Reabsorption of the peripheral oedema that had accumulated while patient was ambulant
- ***Paroxysmal nocturnal dyspnoea (PND)***
  - Severe shortness of breath occurring at night, which awakens the patient from sleep and is relieved by sitting upright
  - It is due to depression of the respiratory centre during sleep and reduced adrenergic stimulation of the myocardium at night resulting in further impairment of myocardial function
- ***Cyanosis*** of mucosa and nail beds
- ***Pulse:*** Sinus tachycardia (due to increased adrenergic activity) and pulsusalternans (alternate large and small volume beats with a normal rhythm)
- ***Blood pressure:*** Diminished pulse pressure due to reduced stroke volume
- ***Jugular venous pressure:*** Raised as a consequence of elevated systemic venous pressure
- ***Liver (congestive hepatomegaly)***
  - Right upper quadrant pain from stretching of the capsule of the liver
  - Liver is enlarged and tender due to elevated right-sided heart pressures transmitted backward into the portal vein circulation
- ***Pleural effusion, ascites and pericardial effusion***
  - Right-sided pleural effusion is more common and results from increased pleural capillary pressure and transudation of fluid into pleural space.
  - Ascites results from transudation secondary to elevated pressure in hepatic veins, portal veins and veins draining the peritoneum.
- ***Acute pulmonary oedema***
  - Caused by marked elevation of pulmonary capillary pressure
  - Clinically, characterized by severe breathlessness, cough with copious, pinkish, frothy expectoration and bilateral crepitations

- Seen over dependent parts (gravity dependent) with sparing of face and arms
  - In ambulatory patients, oedema is seen over the legs, particularly in the pretibial region. It is less in the morning and more towards the evening. Oedema is sacral in bedridden patients.
  - In advanced stages cases, generalized oedema or anasarca may be seen.
- ***Nonspecific symptoms***
  - Anorexia, nausea, abdominal pain and fullness (congestion of liver and portal venous system)
  - Fatigue and weakness (reduced perfusion of skeletal muscles)
  - Low-grade fever (reduced cutaneous flow)

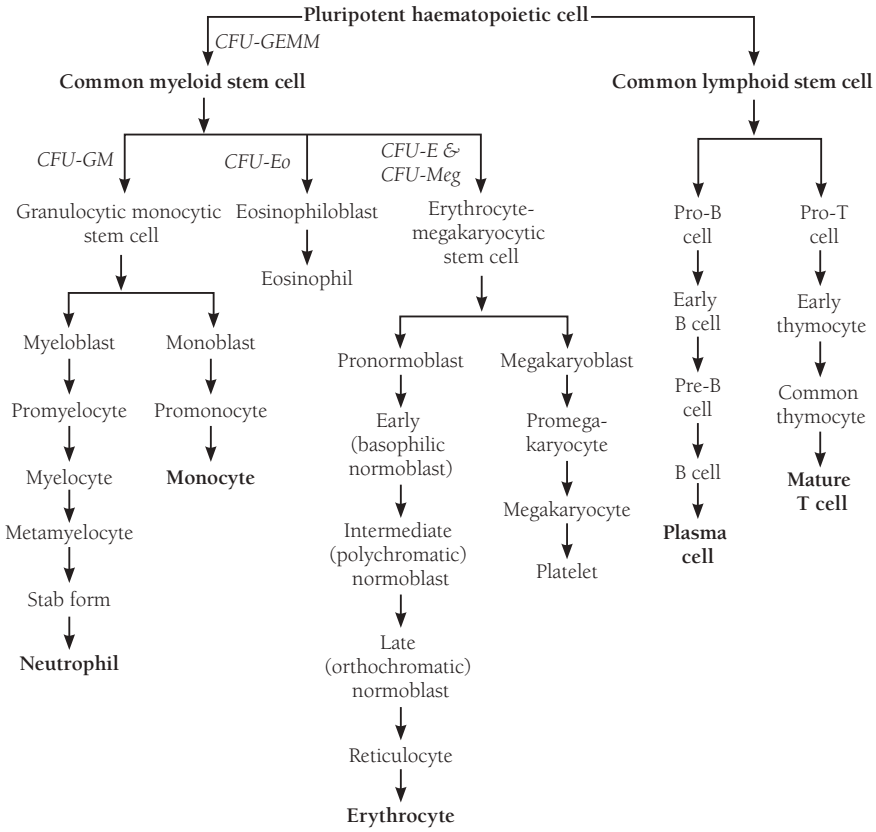
Diagnosis

- Chest radiograph may show cardiomegaly, prominence of upper lobe veins, Kerley A and B lines (cuffing of the areas around the bronchi) and other features of pulmonary oedema.
- ECG to identify arrhythmias, ischaemic heart disease and right and left ventricular hypertrophy
- Echocardiography for evaluation of ejection fraction, valvular status and chamber size.

12

Haematology

Normal Sequence of Development of Cells of Haematopoietic System (Flowchart 12.1)



FLOWCHART 12.1. Normal development of cells of Haematopoietic system.

Normal reference haematological values are listed in Table 12.1.

TABLE 12.1. Normal haematological values

Haemoglobin (Hb)	**Adult male:** 155 ± 25 g/L **Adult female:** 140 ± 25 g/L
Mean corpuscular volume (MCV)	***86 ± 10 fL***
Mean corpuscular haemoglobin (MCH)	***29.5 ± 2.5 pg***
MCH concentration (MCHC)	***325 ± 25 g/L***
RBC diameter	***6.7–7.7 microns***

Continued

TABLE 12.1. Normal haematological values—cont'd

RBC life span	***120 ± 30 days***
RBC count	**Adult male:** ***5.5 ± 1 × 10^{12}/L*** **Adult female:** 4.8 ± 1 × 10^{12}/L
WBC or TLC	***7.5 ± 3.5 × 10^{9}/L***
Differential leukocyte count (DLC)	***Adults:*** Neutrophils 2–7.5 × 10^{9}/L (40–75%) Lymphocytes 1.5–4 × 10/L (20–45%) Monocytes 0.2–0.8 × 10^{9}/L (2–10%) Eosinophils 0.04–0.4 × 10^{9}/L (1–6%) Basophils 0.02–0.1 × 10^{9}/L (1%) ***Children:*** Neutrophils 2–6 × 10^{9}/L Lymphocytes 5.5–8.5 × 10^{9}/L Monocytes 0.7–1.5 × 10^{9}/L Eosinophils 0.3–0.8 × 10^{9}/L Basophils 0.02–0.1 × 10^{9}/L
Platelet count	***150–400 × 10^{9}/L***
Reticulocyte count	**Adults:** ***0.5–2.5%*** **Infants:** ***2–6%***
Erythrocyte sedimentation rate (ESR)	**Westergren (1 h at 20 ± *3°C)*** ***Adult male: 10 mm*** ***Adult female: 15 mm*** ***Children: 10 mm***
Bleeding time (BT)	**Ivy's method:** ***2–7 min*** **Template method:** 2.5–9.5 min
Prothrombin time (PT)	***11–16 s***
Partial thromboplastin time with kaolin (PTTK)	***30–40 s***
Plasma fibrinogen	***1.5–4 g/L***

Q. Write briefly on haematopoietic growth factors.

Ans. Haematopoiesis is regulated by several growth factors.

- **Interleukins (ILs)-1, -3, -6** exert their primary effects early in stem cell differentiation (therefore, important for the differentiation of multiple blood lineages). IL-1 and -2 are B- and T-cell regulators; IL-3 stimulates granulocyte, macrophage, eosinophil and megakaryocyte colonies.
- **Granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF)** and **thrombopoietin (TPO)** exert their effects later in the differentiation cascade, and their effects are more lineage specific.
- **Erythropoietin (EPO)** is a heat-stable glycoprotein having a molecular weight of 36 kD. About 90% EPO is produced in the kidney and the remaining in extrarenal sites like the liver. EPO gene is present on human chromosome 7q21. It is the prime regulator of erythropoiesis. Hypoxia induces the release of EPO from juxtaglomerular cells of the kidney. EPO then binds to receptors on erythroid cells in the bone marrow inducing their proliferation and differentiation.
- **G-CSF** promotes the differentiation of granulocytes and **GM-CSF** causes an increase in neutrophils, eosinophils, macrophages and sometimes lymphocyte counts.
- **TPO** is the most potent cytokine-promoting proliferation and maturation of megakaryocytes.

Q. Discuss the aetiology, classification and clinical features of anaemia.

Ans. Anaemia is defined as a state in which the blood haemoglobin level is below the normal range for the patient's age and sex (Table 12.2).

TABLE 12.2. Anaemic ranges of haemoglobin

Age/sex (years)	Hb (g/dL)
Children (0.5–4)	< 11.0
Children (5–12)	< 11.5
Children (12–15)	< 12.0
Adult men	< 13.0
Nonpregnant women	< 12.0
Pregnant women	< 11.0 (the lower limit is reduced in pregnancy)

Anaemia is graded into mild, moderate and severe based on the haemoglobin value and clinical presentation (Table 12.3).

TABLE 12.3. Severity of anaemia

Severity	Hb range (g/dL)
Mild	9.5–13.0 (Often no signs or symptoms; commonly remains untreated)
Moderate	8.0–9.5 (May present with symptoms; requires management to prevent development of complications)
Severe	< 8.0 (Symptoms usually present; may be life-threatening and requires prompt management)

Classification of Anaemias

Anaemias are classified based on

1. The cause of anaemia **(aetiological classification)**
2. The morphology of red cells **(morphological classification)**

1. **Aetiological classification of anaemia**
   (a) **Anaemia of blood loss (may be acute or chronic):**
      (i) **Acute:** Trauma, major surgical procedures and postpartum bleeding
      (ii) **Chronic:** Hookworm infestation, bleeding peptic ulcer, carcinoma colon, IBD, haemorrhoids and excessive menstrual loss
   (b) **Decreased production of red cells:**
      (i) **Nutritional deficiency:** Iron (affects haemoglobin synthesis), vitamin B_{12} and folate (affect DNA synthesis)
      (ii) **Inherited genetic defects:**
         - Defects leading to stem cell depletion (Fanconi anaemia)
         - Defects affecting erythroblast maturation (Thalassaemia syndromes)
      (iii) **Erythropoietin deficiency:** Renal failure and anaemia of chronic disease
      (iv) **Immune-mediated injury of progenitors:** Hypoplastic or aplastic anaemia and pure red cell aplasia
      (v) **Marrow invasion:** Leukaemia, lymphoma, secondary carcinoma and granulomatous disease
      (vi) **Inflammatory iron sequestration:** Anaemia of chronic disease.
      (vii) **Unknown mechanisms:** Endocrine disorders (Hypothyroidism, hypoadrenalism and hypopituitarism) and hepatic disease
   (c) **Due to excessive destruction of red cells (haemolytic anaemias):**
      (i) **Genetic disorders:** Red cell membrane, enzyme abnormalities, haemoglobinopathies, like sickle cell disease and thalassaemias
      (ii) **Acquired disorders:** Immune, toxic, mechanical and infectious causes

2. **Morphological classification of anaemia**
   (a) Microcytic hypochromic anaemia (MCV < 80 fL; MCH < 27 pg)
   (b) Macrocytic normochromic anaemia (MCV >100 fL; MCH within normal range)
   (c) Normocytic normochromic anaemia (MCV 80–100 fL; MCH within normal range)

Symptoms of Anaemia

Acute:
Shortness of breath, organ failure and shock
Chronic:

1. *General symptoms (due to tissue hypoxia)*
   - Fatigue, lassitude, dyspnoea, palpitations, dizziness, headache, syncope, tinnitus and vertigo
   - Irritability, sleep disturbances, lack of concentration and paraesthesias
   - Anorexia, nausea and bowel disturbances
   - Symptoms of cardiac failure
   - Amenorrhea and polymenorrhea
2. *With haemolysis*: Skeletal abnormalities (due to expansion of marrow), growth retardation, jaundice and gallstones
3. *With defective erythropoiesis*: Iron overload leading to heart and endocrine failure

Signs of Anaemia

- Pallor of skin and mucous membranes, nail beds and palpebral conjunctivae
- Tachycardia with a wide pulse pressure, ejection systolic murmur; best heard over the pulmonary area
- Cardiac dilatation and later, signs of cardiac failure
- Oedema

Morphological abnormalities of red cells in different types of anaemias are described in Table 12.4.

TABLE 12.4. Morphological abnormalities of red cells in different types of anaemias

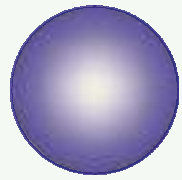
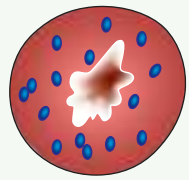
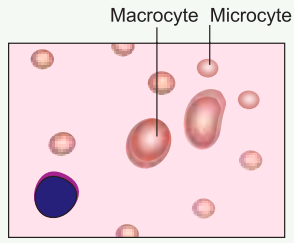
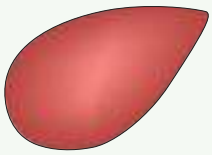
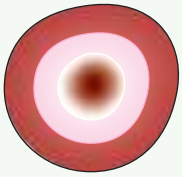
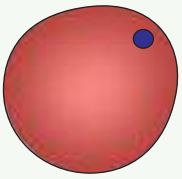
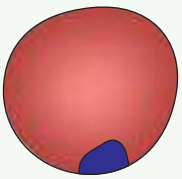
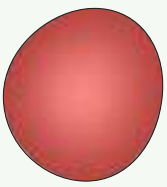
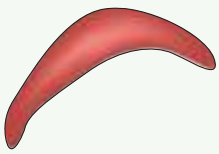
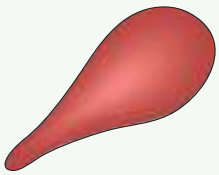
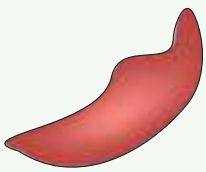
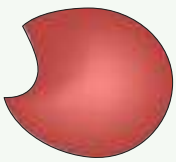
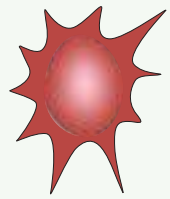
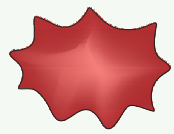
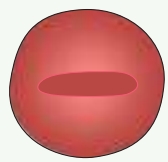
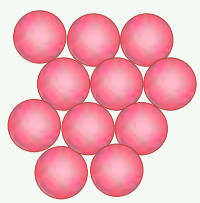
Abnormalities	Description	Morphology
Polychromatophilia or polychromasia	Red cells staining bluish-grey; they represent young reticulocytes and are seen in the conditions associated with accelerated erythropoiesis	
Punctate basophilia or basophilic stippling	Presence of fine or coarse purple-blue granules in the cytoplasm of red cells, representing ribosomal aggregates **Causes**: β-Thalassaemia, haemoglobinopathies, lead and arsenic poisoning	
Anisocytosis ***Microcytes***	Variation in size of the cell as seen in iron deficiency Small red cells (MCV < 80 fL) with decreased amount of haemoglobin. **Causes:** Iron deficiency anaemia, thalassaemia, anaemia of chronic disease, lead poisoning and sideroblastic anaemia	
Macrocytes	Large red cells (MCV > 100 fL; size > 8.5 microns, normal MCH). May be round or oval **Causes:** Impaired DNA synthesis (B_{12} and folate deficiency), accelerated erythropoiesis, excessive surface membrane (liver disease or splenectomy)	

TABLE 12.4. Morphological abnormalities of red cells in different types of anaemias—cont'd

Abnormalities	Description	Morphology
Poikilocytosis	Variation in shape of the cell as seen in thalassaemias	
Target cells (codocytes)	Flat red cells with a central mass of haemoglobin (dense area), surrounded by a ring of pallor (pale area) and an outer ring of haemoglobin (dense area) **Causes**: Iron deficiency, chronic liver disease, hyposplenism and haemoglobinopathies	
Howell–Jolly bodies	Remnants of nuclear material left after the nucleus is extruded, these are normally removed by spleen **Causes:** Nonfunctioning or absent spleen and megaloblastic anaemia	
Heinz bodies	These are formed from denatured aggregated haemoglobin. Cells containing Heinz bodies are normally removed by spleen. They do not stain with Romanowski stains but can be demonstrated by Supravital stains like new methylene blue. **Causes:** Thalassaemia, asplenia and chronic liver disease	
Spherocytes	Dark appearing (densely haemoglobinized) red cells with no central pallor (normal or decreased MCV and increased MCHC) **Causes:** Immune haemolytic anaemia, haemolytic disease of newborn, burns and hereditary spherocytosis	
Sickle cells (drepanocytes)	Curved red cells with pointed ends **Causes:** Sickle-cell anaemia, HbS-β Thalassaemia	
Teardrop cells (dacrocytes)	Red cells in the shape of teardrops; they are pathological and should be followed up **Causes:** Infiltrative disorders of bone marrow (eg, myelofibrosis); may be seen in megaloblastic anaemia, β Thalassaemia, renal failure and tuberculosis	
Schistocytes	Fragmented red cells **Causes:** Mechanical stress, eg, microangiopathic haemolytic anaemia, thermal injury and severe burns	
Bite cells	Red cells with peripheral single or multiple arcuate defects (bites) **Causes:** Red cells enzyme defects (G-6-PD deficiency and Pyruvate kinase deficiency)	

Continued

TABLE 12.4. Morphological abnormalities of red cells in different types of anaemias—cont'd

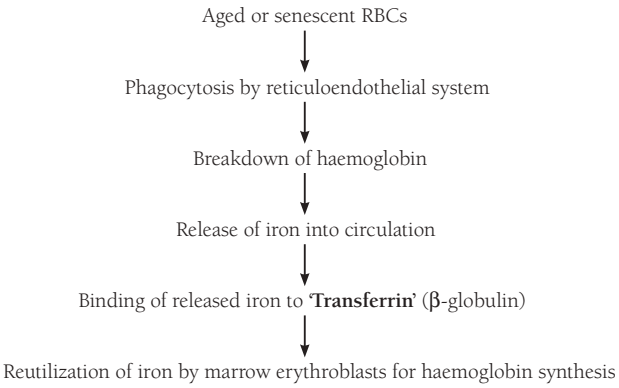
Abnormalities	Description	Morphology
Acanthocytes (spur or spicule cells)	Spheroidal red cells with a few spiny/thorny projections (5–10 in number with irregular thickness and spacing) **Causes:** Post-splenectomy, alcoholic cirrhosis, pyruvate kinase deficiency, autoimmune haemolytic anaemia (AIHA), severe burns and renal disease	
Echinocytes (Burr or sea urchin cells)	Small cells or cell fragments bearing shorter, more regular spines 10–30 in number, evenly spaced **Causes:** Uraemia, pyruvate kinase deficiency, blood storage, ATP depletion, calcium accumulation and contact with glass	
Elliptocytes	Elliptical cells **Causes:** Hereditary elliptocytosis, thalassaemia, sickle cell trait, iron deficiency, megaloblastic and myelophthisic anaemia	
Stomatocytes	Uniconcave red cells with a slit-like area of central pallor **Causes:** Hereditary stomatocytosis, alcoholism, cirrhosis, obstructive liver disease	
Agglutination	True agglutination is irregular clumping of red cells into grape like clusters (in contrast with pseudoagglutination or rouleaux formation which is observed in paraproteinaemias, hypergammaglobulinaemia and fibrinogenaemia which causes stacking of red cells like coins)	

Q. Write in detail on iron metabolism.

Ans. Salient features pertaining to iron metabolism in human body:

- **Average daily intake of iron in a normal adult:** 10–20 mg; 10% (1-2 mg) of which is usually absorbed.
- **Chief dietary sources of iron:** Meat, liver, kidney, egg yolk, green leafy vegetables and fruits; milk is a poor source.
- **Dietary iron:** Two major types:
  Haem iron (found in animal products and more readily absorbed)
  Nonhaem iron (found as inorganic iron in vegetables, less readily absorbed)
- **Total body iron:** 3–5 g (proportionate to body weight)
- Eighty percent of functional iron is in **haemoglobin**: 2–3 g
- **Storage or available tissue iron** (ferritin and hemosiderin): 1 g
- **Essential or nonavailable iron** (myoglobin and other enzymes of cellular respiration): 0.5 g
- Iron is transported in the plasma, bound to a glycoprotein called **transferrin (plasma or transport iron):** 0.003–0.004 g

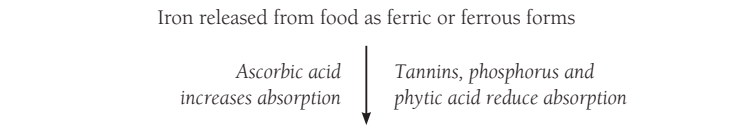
Fate of Haemoglobin Iron (Flowchart 12.2)



FLOWCHART 12.2. Fate of Haemoglobin Iron.

1. ***Released iron is also converted into:***
   - Storage or available iron
   - Nonavailable iron
2. ***A small proportion of storage iron is released into the plasma.***
3. ***A small proportion is lost in urine, sweat, faeces or blood.***

Iron Absorption (Flowchart 12.3)



FLOWCHART 12.3. Mechanism of Iron absorption.

Proximal intestine is the site of choice because it has the ideal pH (redox potential for iron absorption) and the key proteins responsible for iron absorption are maximally expressed in this part. A ferric reductase enzyme **duodenal cytochrome b (dcytb) reductase**, expressed on the enterocyte brush border, reduces ferric to ferrous form to make it a substrate for transport by **Divalent Metal Transporter (DMT) 1.**

Mucosal uptake of haem and nonhaem iron occurs through two distinct pathways:

1. **Nonhaem iron:** Enters the apical and basolateral portions of villus enterocytes by the action of transporter protein **DMT1.** Inside the cell, most of the iron is stored as mucosal ferritin and not absorbed. These ferritin-containing cells are later exfoliated from the mucosal surface into the intestine at the end of their 3–4-day lifespan. The remaining iron is transported to plasma by **ferroportin (IREG-1 transporter)** and **hephaestin (an iron oxidase)**.
2. **Haem iron:** Mechanism not well understood but it is thought that haem enters the mucosal surface unchanged to release iron within the cells by the action of the enzyme **haem oxygenase**. Iron from this source follows the same pathway as nonhaem iron except that a small portion of the haem iron passes into the plasma intact to bind to hemopexin, a haem-binding protein.

Storage and Transport of Iron

- Iron is stored in bone marrow, liver, spleen and skeletal muscle as ferritin and haemosiderin.
- Ferritin has a spherical outer shell of protein called **apoferritin** and an inner core of **trivalent iron.**
- Haemosiderin is more stable, therefore less readily mobilized to haemoglobin formation.

- Percentage of total iron-binding protein to which iron is attached is called percentage saturation. Normal percentage saturation is 33%.

$$\left\{\%\text{age saturation} = \frac{\text{Serum Fe}}{\text{TIBC}}\right\}$$

Iron Studies

- Serum iron levels in a normal adult are 0.7–1.8 mg/L.
- Normal TIBC (total iron-binding capacity) is 2.5–4.0 mg/L.
- Normal ferritin levels are 20–300 mcg/L in males and 15–150 mcg/L in females.
- Ferritin levels are raised in acute leukaemias, inflammation and Hodgkin disease.
- Ferritin levels are decreased in liver disease.

Iron Excretion

Normal average excretion of iron in urine, faeces and sweat per day is about 0.5–1 mg. Another 0.5–1.0 mg is lost in menses.

Q. Outline the aetiopathogenesis, clinical features and blood picture of iron-deficiency anaemia (IDA).

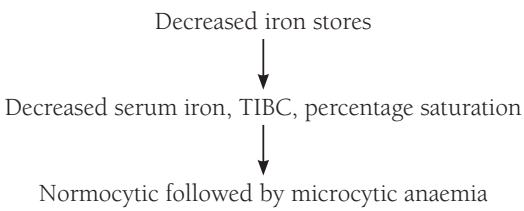
Ans. IDA is the most common type of anaemia met with in clinical practice.

Causes

- **Pathological blood loss:** Peptic ulcer, haemorrhoids, hiatus hernia, carcinoma stomach and colon, chronic aspirin ingestion, oesophageal varices, ulcerative colitis, hookworm infestation, haematuria, repeated epistaxis, haemoptysis and pathological uterine bleeding.
- **Increased physiological demand:** Growing children and women in reproductive age group
- **Inadequate intake**
  - **Nutritional deficiency:** Poverty, dietary fads, anorexia and poor bioavailability of nonhaem iron.
  - **Impaired absorption:** Gastroenterostomy, tropical sprue, celiac disease, atransferrinaemia, abnormal transferring function or antibodies to transferring receptors.
  - **Plummer–Vinson (Paterson–Kelly) syndrome:** Syndrome complex of chronic iron deficiency, dysphagia due to postcricoid web and glossitis

Stages of Iron Deficiency

1. Storage iron depletion
2. Iron-deficient erythropoiesis
3. Frank iron-deficiency anaemia



Clinical Features

***Signs and symptoms are due to*:**

- **Anaemia:** Lassitude, weakness, fatigue, dyspnoea, palpitations, angina, CCF and pallor
- **Epithelial tissue changes:**
  - Nails: Thin, lusterless, brittle, show ridging and flattening; presence of koilonychia (spoon-shaped nails)

- Tongue: Atrophy of papillae, shiny or glazed tongue, glossitis and angular stomatitis
- Plummer–Vinson (Paterson–Kelly) syndrome: Characterized by chronic iron deficiency, dysphagia and glossitis; seen in middle-aged to elderly women who have chronic iron deficiency and a fine web or band composed of desquamating epithelial cells at the oesophageal entrance (postcricoid web). These patients present with dysphagia to solids.

- **Pica:** This is defined as a craving to eat substances like dirt, clay, salt, hair and is a typical manifestation of iron deficiency.
- **Recurrent infections:** Iron deficiency induces defective lymphocyte-mediated immunity and impairs bacterial killing by phagocytes leading to impaired immunity and recurrent infections.

Laboratory Diagnosis

1. **General blood parameters**
   (a) **Hb:** Decreased
   (b) **RBC count:** Decreased
   (c) **RBC indices:** Reduced/low
2. **Peripheral smear (**Fig. 12.1**)**
   (a) **Microcytic hypochromic cells** (red cells are smaller than normal and have increased central pallor)
   (b) **Anisocytosis or variation in cell size** (anisocytosis is indicated by increased **red cell distribution width (RDW)** and is more marked in IDA than in thalassemia for the same haemoglobin value)
   (c) **Poikilocytosis or variation in cell shape** (less marked in IDA than in thalassaemia for the same haemoglobin value)
   (d) **Normoblasts, elliptocytes, pencil-shaped cells** and **target cells** (common in severe anaemia)
   (e) Normal, increased or decreased **platelet count** and unremarkable **WBCs**
3. **Reticulocyte count:** Normal or decreased (in post-haemorrhagic anaemia reticulocyte count may be mildly raised)
4. **Bone marrow**
   (a) Presence of erythroid hyperplasia; increase mainly in mature cells
   (b) Predominant cell is a polychromatic normoblast, which is smaller than normal (micronormoblast)
   (c) Cytoplasm shows ragged borders

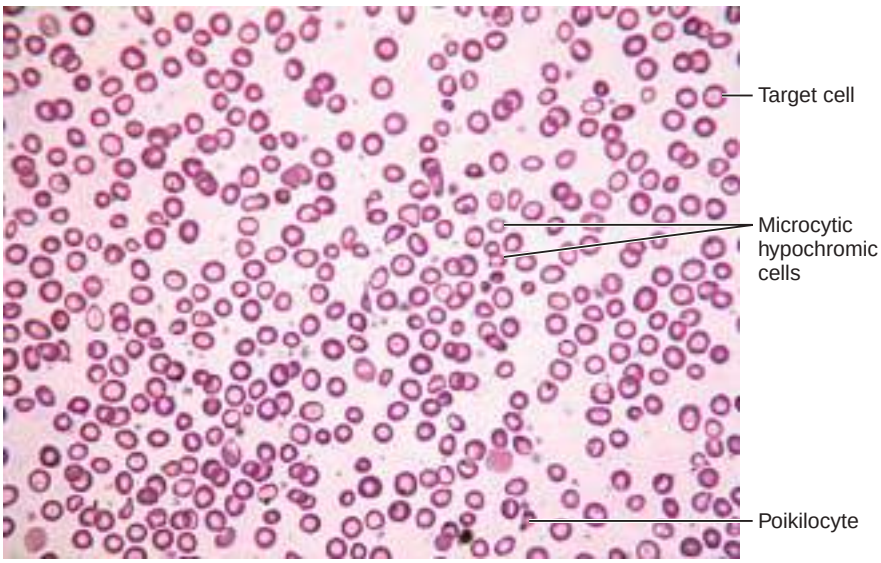


FIGURE 12.1. Leishman-stained PBS of iron deficiency anaemia showing marked hypochromia and anisocytosis with the presence of a fair number of microcytes.

(d) Cytoplasmic maturation lags behind that of the nucleus (due to delayed haemoglobinization)
(e) Prussian blue stain shows decreased iron stores

5. **Iron studies**
   (a) Decreased serum iron
   (b) Percentage saturation < 16%
   (c) Serum ferritin < 12 mcg/L (most useful)
   (d) Serum transferrin receptor fragments assay (sTFR) by immunological methods demonstrates an increased value in IDA
   (e) Erythrocyte ferritin is decreased in iron deficiency and free erythrocyte zinc protoporphyrin (FEP) levels are increased in disorders of haem synthesis including iron deficiency. Normally protoporphyrin binds iron to form haem in erythroid precursors. In IDA protoporphyrin fails to bind iron and its levels increase. In thalassemia FEP levels are normal.

Differential Diagnosis

- **Heterozygous form of β-thalassaemia/β-thalassaemia trait**
  - RBC count normal to increased (decreased in iron-deficiency anaemia)
  - MCV and MCH disproportionately lower than degree of anaemia
  - RDW normal in thalassaemia (increased in iron-deficiency anaemia)
  - Target cells abundant (few in iron-deficiency anaemia)
  - Basophilic stippling and nucleated RBCs commonly seen (not seen or rare in iron deficiency)
  - Normal iron studies (deranged values in iron deficiency)
  - Increased HbF (normal in iron deficiency)
- **Anaemia of chronic disorders**
  - Serum iron decreased
  - Serum ferritin normal or increased (decreased in iron deficiency)
  - TIBC normal or decreased (increased in iron deficiency)
  - Percentage saturation decreased to normal (decreased in iron deficiency)
- **Sideroblastic anaemia**
  - Serum iron normal or increased (decreased in iron deficiency)
  - Serum ferritin normal or increased (decreased in iron deficiency)
  - TIBC normal (increased in iron deficiency)
  - Percentage saturation normal or increased (decreased in iron deficiency)

Q. Write briefly on sideroblastic anaemias.

Ans. The sideroblastic anaemias are inherited or acquired disorders characterized by:

- Refractory anaemia with a variable number of hypochromic red cells in peripheral smear
- Excess of iron and ring sideroblasts in the marrow

Classification

1. **Hereditary sideroblastic anaemia**
   X-linked disorder

2. **Acquired sideroblastic anaemia**
   (a) Idiopathic or primary (a type of myelodysplasia):
      (i) Refractory anaemia with ring sideroblasts
      (ii) Refractory anaemia with ring sideroblasts and thrombocytosis
      (iii) Refractory anaemia with multilineage dysplasia and ring sideroblasts
   (b) Secondary, which may be due to:
      (i) *Drugs,* eg, isoniazid, alcohol abuse and lead toxicity
      (ii) *Haematological disorders,* eg, acute leukaemia, lymphoma, haemolytic anaemia, myelofibrosis and polycythaemia vera
      (iii) *Others,* eg, hypothermia, rheumatoid arthritis and carcinoma

Characteristic Features

- Affects middle-aged to elderly individuals who present with insidious onset moderate to severe anaemia.
- The peripheral smear shows a **dimorphic picture** with **microcytic, hypochromic** and **normocytic cells.**
- Iron overload with characteristic **ringed sideroblasts** are seen in the bone marrow (iron enters mitochondria surrounding the nucleus, cannot exit and appears as '**rings**' with Prussian blue staining).
- Siderocytes called **Pappenheimer bodies** (containing nonhaem iron granules) are seen in the RBCs.
- Bone marrow shows erythroid hyperplasia and ineffective erythropoiesis. Dyserythropoiesis is common.
- There is marked increase in serum iron and transferrin saturation.

Q. Enumerate the causes of microcytic hypochromic anaemia.

Ans. ***Causes of microcytic hypochromic anaemia include***

- Iron-deficiency anaemia
- Thalassaemia syndromes
- Sideroblastic anaemia
- Chronic lead poisoning
- Some cases of anaemia associated with chronic disorders

Q. Enumerate the salient features of hypochromic anaemias.

Ans. Salient features of different types of hypochromic anaemias are summarized in Table 12.5.

TABLE 12.5. Salient features of different hypochromic anaemias

Features	Iron deficiency anaemia	Thalassaemia	Sideroblastic anaemia	Anaemia of chronic disorders
Serum iron	Decreased	Normal or increased	Increased	Decreased
TIBC	Raised	Normal	Normal	Decreased
Percent saturation	Decreased	Normal to increased	Normal to increased	Decreased to normal
Serum ferritin	Decreased	Normal	Normal	Normal to increased
Marrow iron stores	Absent	Present	Present	Present
Iron in normoblasts	Absent	Present	Ring sideroblasts	Absent
Hb electrophoresis	Normal	Abnormal	Normal	Normal
Red cell indices	Reduced	Very low	Low	Low normal to reduced
RDW	High	Normal	Normal	Normal
Diagnostic feature/ investigation	Decreased serum ferritin	Hb electrophoresis	Presence of ring sideroblasts	Presence of normocytic population

Q. Describe the aetiopathogenesis, clinical features and haematologic picture of macrocytic anaemia.

Ans. A **macrocyte** is defined as a large red cell with increased volume (**MCV** > 100 fL), diameter (> 8.5 μL) and thickness (**MCH** >31.5 pg; **MCHC** normal). Increased thickness is perceived as a loss of central pallor.

A **megaloblast** is so labelled when a large erythroid cell shows nuclear-cytoplasmic asynchrony (maturation of nucleus lags behind maturation of cytoplasm due to impaired DNA synthesis caused by the deficiency of vitamin B_{12} and folate).

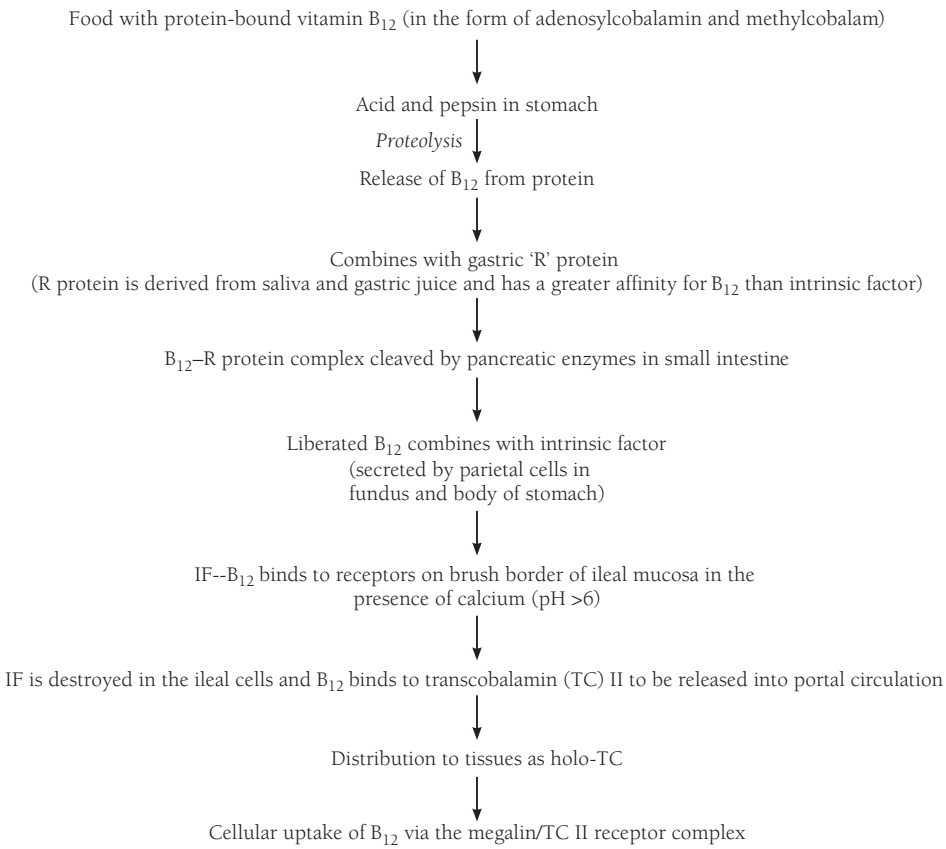
The salient features pertaining to metabolism of vitamin B_{12} and folate are listed in Table 12.6.

TABLE 12.6. Salient features pertaining to the metabolism of vitamin B_{12} and folate

Features	B_{12}	Folate
Content in food	Vegetables: poor source Meat: rich source Milk: good source	Vegetables: rich source Meat: moderate source Milk: poor source
Effect of cooking	10–30% loss	60–90% loss
Adult: daily requirement	2–4 mcg	200 mcg
Adult: daily intake	5–30 mcg	100–500 mcg
Site of absorption	Ileum	Duodenum and jejunum
Body stores	2–5 mg Adequate for 3 years	5–20 mg Adequate for 4 months

Vitamin B_{12}

1. ***Dietary sources of vitamin B_{12}***: B_{12} is predominantly present in animal products (meat, muscle, fish, eggs, cheese and milk); therefore, pure vegetarians may suffer from deficiency. B_{12} is synthesized by bacteria in nature. In humans, these bacteria are normal inhabitants of large bowel but B_{12} cannot be absorbed from this site and thus humans are entirely dependent on dietary sources.
2. ***Absorption of B_{12}***: May be
   (a) Active (Flowchart 12.4)
      (i) Responsible for absorption of physiological amounts of vitamin B_{12}
      (ii) Highly efficient but slow mechanism (takes 8–12 h from ingestion to attainment of peak levels in blood)



FLOWCHART 12.4. Absorption of vitamin B_{12} by active mechanism.

(b) Passive mechanism
  (i) No carrier molecule involved
  (ii) Seen with supraphysiological doses of vitamin B_{12}
  (iii) Absorption occurs through the buccal, gastric and jejuna mucosa

3. ***Transportation of Vitamin B_{12}***: **Cobalamin** is a general term for compounds with biologic vitamin B_{12} activity. These compounds are involved in nucleic acid metabolism, methyl transfer, myelin synthesis and repair. They are necessary for the formation of normal RBCs. Cobalmin has a transport form (methylcobalamin) and a storage form (adenosylcobalamin). There are three major vitamin B_{12}-binding proteins in plasma namely transcobalamins I, II and III.

Transcobalamin I (Haptocorrin)
- α-1 Globulin synthesized by granulocytes
- 70–80% of endogenous B_{12} is bound to TC I
- Required for storage and not essential for transport; therefore, its absence does not lead to clinical signs of B_{12} deficiency

Transcobalamin II
- β-Globulin synthesized in liver
- Essential for transport of B_{12} from organ to organ and from cell to cell (B_{12} bound to TC II is known as holotranscobalmin or holo-TC)
- Deficiency leads to severe megaloblastic anaemia

Transcobalamin III
- Binds a small amount of B_{12}

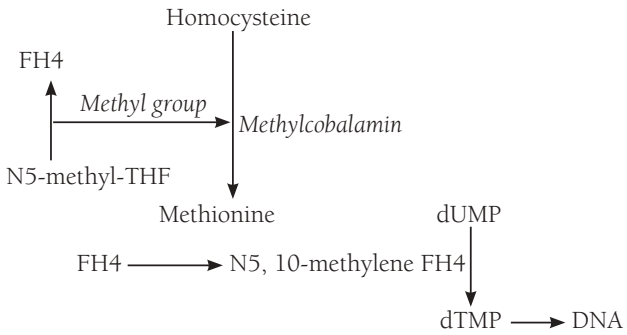
4. ***Storage of B_{12}***
  (a) The liver stores large amounts of vitamin B_{12} followed by the kidneys, heart and brain.
  (b) B_{12} is excreted through bile and shedding of intestinal epithelial cells (enterohepatic reabsorption helps to retain vitamin B_{12}).

5. ***Functions of B_{12}***

(a) Methylmalonyl CoA
↓
- *Methylmalonyl CoA mutase*
- *Adenosylcobalamin (B_{12})*

Succinyl CoA

(b)



Absorbed N5-methyl FH4 gives away a methyl group to synthesize methionine from homocysteine in a step requiring cobalamin and generates FH4 (tetrahydrofolate), which is reconjugated to N5, 10-methylene FH4 for use in thymidylate and purine synthesis.

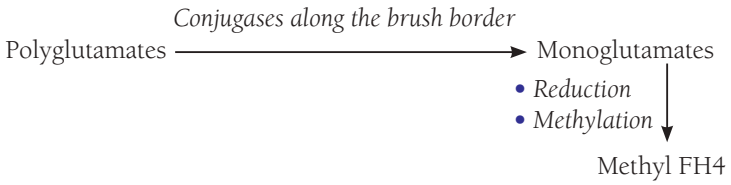
6. ***Deficiency of B_{12}***: Leads to

(a) Increased levels of methylmalonate and propionate
↓
Synthesis of abnormal myelin lipids
↓
Myelin degeneration and neurological abnormalities

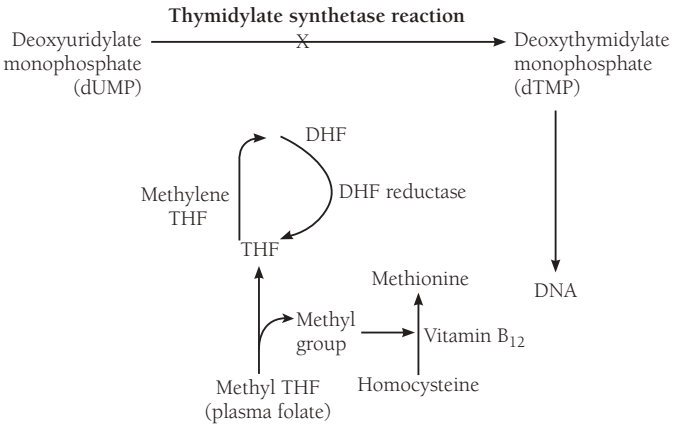
(b) Impaired DNA synthesis and trapping of folate as methyltetrahydrofolate (FH4)

Folate

1. ***Dietary sources of folate*:** Green leafy vegetables are a rich source of folate. Moderate amounts are present in meat, and milk is a poor source.
2. ***Absorption, transport and storage of folate*:**
   (a) Folate is a yellow compound with a chemical name pteroylglutamic acid (PGA).
   (b) It exists in nature as a polyglutamate (conjugated folate).
   (c) For its action as a coenzyme, it must be converted to dihydro or tetrahydro form.
   (d) Folate is absorbed in the proximal jejunum and ileum; the mechanism of absorption, however, is unclear.



   (e) Folate circulates free or bound to albumin in the plasma as N5-methyl FH4.
   (f) Storage in liver is in polyglutamate form.
3. ***Functions of folate*:**
   (a) Folates act as 1-carbon unit carriers and are needed for synthesizing DNA and RNA, as well as the conversion of homocysteine to methionine (Flowchart 12.5).



X–Block due to folate deficiency.

FLOWCHART 12.5. Role of B_{12} and folate in DNA synthesis.

   (b) Synthesis of purines
   (c) Histidine metabolism (deficiency of folate leads to increased formiminoglutamic acid or FIGLU)

Macrocytic Anaemia

Macrocytosis may be
- Megaloblastic (with impaired DNA synthesis)
- Nonmegaloblastic (with normal DNA synthesis)

Causes of Megaloblastic Macrocytosis

1. Deficiency of vitamin B_{12} and/or folate
2. Resistance to B_{12} or folic acid therapy due to metabolic inhibitors of DNA synthesis or folate metabolism

Causes of Vitamin B_{12} Deficiency

- **Decreased intake:** Nutritional deficiency (vegans, breastfed infants of vegan mothers)
- **Impaired absorption:**
  - **Gastric causes:** Pernicious anaemia, destruction of gastric mucosa or gastric bypass surgery
  - **Intestinal causes:** Malabsorption due to enteritis, celiac disease or tropical sprue, competition for vitamin B_{12} in fish tapeworm (*Diphyllobothriumlatum*) infestation or blind loop syndrome (bacterial overgrowth in diverticulae of bowel)
  - **Drug-induced malabsorption:** Implicated drugs include PAS, colchicine, neomycin, ethanol and KCL
  - **Chronic pancreatic disease:** Lack of pancreatic proteases and inability to degrade R proteins, which compete with IF
  - **Zollinger–Ellison syndrome:** Impaired absorption due to low pH of intestinal contents reaching ileum
  - **Haemodialysis:** Cause unknown

Causes of Folate Deficiency

- **Inadequate intake:** Young persons on junk food diets, elderly and terminally ill people
- **Inappropriate cooking methods:** Polyglutamates are sensitive to heat; boiling, steaming or frying the food destroys folate content
- **Excess utilization**: Pregnancy, haemolysis and tumours
- **Alcoholism:** Reduces serum folate levels are attributed to inadequate diet, excessive urinary loss and interference with the enterohepatic circulation of folate by alcohol.
- **Impaired absorption**
  - Celiac disease and tropical sprue
  - Drugs that block dihydrofolate reductase (methotrexate and trimethoprim), block conversion of polyglutamates to monoglutamates (phenytoin), decrease absorption and increase metabolism (anticonvulsants) and decrease absorption and increase urinary excretion (oral contraceptives)
- **Complication of haematological illness:** Increased demand due to rapid proliferation of haematopoietic cells in haemolytic anaemia, PNH, myelofibrosis, sideroblastic anaemia, leukaemia and multiple myeloma.

Causes of Nonmegaloblastic Macrocytic Anaemia

- **Haemolytic and posthaemorrhagic anaemia:** Result in accelerated erythropoiesis, which leads to increased reticulocyte count, premature release of the bone marrow reticulocytes and shortened time between all cell divisions/skipping of cell division, all of which cause macrocytosis.
- **Thin macrocytosis:** Thin macrocytes typically have increased surface area to volume ratio. Increased surface area is attributed to excessive lipid content which in turn may be seen in:
  - **Hepatic disease (obstructive jaundice):** ↓ Bile salt excretion →↑ Bile salt in plasma →↑ Free cholesterol due to decreased esterification → Increased uptake of cholesterol by RBCs → Increased membrane surface area
  - **Postsplenectomy state:** During maturation of reticulocytes in spleen, there is loss of lipids; in the absence of spleen, there is decreased loss and excessive accumulation of lipids in the RBC membrane resulting in increased surface area.
- **Myelodysplastic syndrome (MDS), eg**
  - Aplastic anaemia
  - 5q-refractory anaemia syndrome
  - Acquired sideroblastic anaemia
  - Hereditary dyserythropoietic anaemia
- **Miscellaneous**
  - Alcoholism
  - Hypothyroidism
  - Myelophthisic anaemia

Clinical Features of Vitamin B_{12} Deficiency

- **General signs and symptoms of anaemia:** Weight loss, angular cheilosis, dermatitis, osteomalacia, pallor, icterus (lemon tint), low-grade fever (in severe anaemia), mucocutaneous bleeding (with thrombocytopenia)

- **Neurological manifestations:** Vitamin B_{12} deficiency causes **sensorimotor demyelinating peripheral neuropathy** (leading to paraesthesias and numbness) and **cerebral changes** (leading to dementia, psychosis or personality changes). This can be accompanied by involvement of **pyramidal tracts** (causing spastic paraparesis, cerebellar dysfunction and optic neuropathy). Vitamin B_{12} is required for transmethylation reactions, which are essential for myelin synthesis. B_{12} deficiency therefore affects white matter of dorsal/posterior and lateral columns of spinal cord leading to sensory ataxia and loss of position and vibration sense. Involvement of multiple pathways is labelled as '**subacute combined degeneration of the spinal cord**'.
- **Splenomegaly and hepatomegaly:** Mild and nontender
- **Gastrointestinal symptoms:** Weight loss and poorly localized abdominal pain
- **Glossitis:** Loss of papillae leading to a smooth beefy red tongue
- **Skin and hair changes:** Premature greying of hair and melanin pigmentation of skin with sparing of mucosa
- **Infertility:** Reversible with correction of deficiency

Clinical Features of Folate Deficiency

Folate deficiency mainly manifests with **megaloblastic anaemia** and **glossitis**. **Subacute combined degeneration is not seen** and **peripheral neuropathy is rare**.

Laboratory Diagnosis of Megaloblastic Anaemia

- **General blood parameters**
  - RBC count and haemoglobin levels are decreased.
  - MCV is increased (>100 fL) and MCH is decreased (less than 33 pg).
  - Reticulocyte count is normal.
- **Peripheral smear** (Fig. 12.2A **and** B)
  - Red cells show anisopoikilocytosis with the presence of **macrocytes** and **macroovalocytes** (large oval RBCs).
  - Also present are **Howell–Jolly bodies** (nuclear remnants left after the nucleus is extruded) and **Cabot rings** (abnormal histone synthesis causes arginine-rich histones to accumulate as rings in red cells).
  - **Neutrophil hypersegmentation** is seen which is defined as greater than 5% neutrophils having more than five lobes or presence of at least one six lobed cell. This is the first haematological abnormality to be seen in megaloblastic anaemia and is thought to be due to decreased production and a compensatory prolonged lifespan of circulating neutrophils (senile neutrophils).
- **Bone marrow**
  - Shows megaloblastic hyperplasia. Nuclei of erythroblasts are large with fine and open sieve-like chromatin. Haemoglobinization of the cytoplasm proceeds at a normal rate; whereas, nuclear maturation lags behind that of the cytoplasm (compared with iron deficiency anaemia in which the cytoplasmic maturation lags behind that of the nucleus). This is called nuclear-cytoplasmic asynchrony.
  - Giant metamyelocytes and stab forms are seen.
  - Megakaryocytes may be large and abnormal.
- **Biochemical tests**
  - **Serum vitamin B_{12}** levels <200 pg/mL indicate vitamin B_{12} deficiency (normal 200–900 pg/mL) and **serum folate levels** <6 ng/mL indicate folate deficiency (normal 6–12 ng/mL). There are two methods to measure serum B_{12}—microbiological and radioisotope assay. The latter is the preferred method (as it is rapid and simple and not affected by presence of antibiotics). Serum B_{12} assay should however be interpreted with caution as it represents the total and not metabolically active B_{12}; is a late biomarker of megaloblastic anaemia and lacks specificity and sensitivity.
  - **Holotranscobalamin (holo-TC)** is considered active B12 and is the earliest biomarker for B_{12} deficiency.
  - **Elevated methylmalonic acid (MMA) level indicates depletion of B_{12} stores.**
  - **Isolated decreased levels of holo TC** supports vitamin B_{12} deficiency and **a combination of decreased holoTc** and **increased MMA** (reference range: 0.08–0.28) and **homocysteine** indicate a metabolically manifest B_{12} deficiency. Increased MMA levels

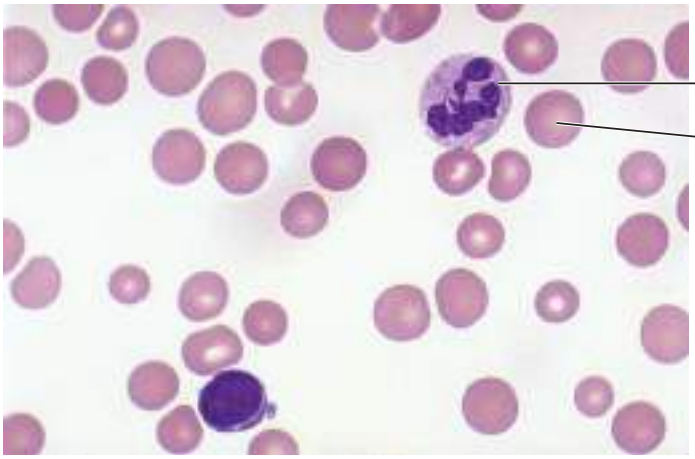


FIGURE 12.2A. Leishman-stained PBS of macrocytic anaemia showing numerous macrocytes and a hypersegmented neutrophil (arrow).

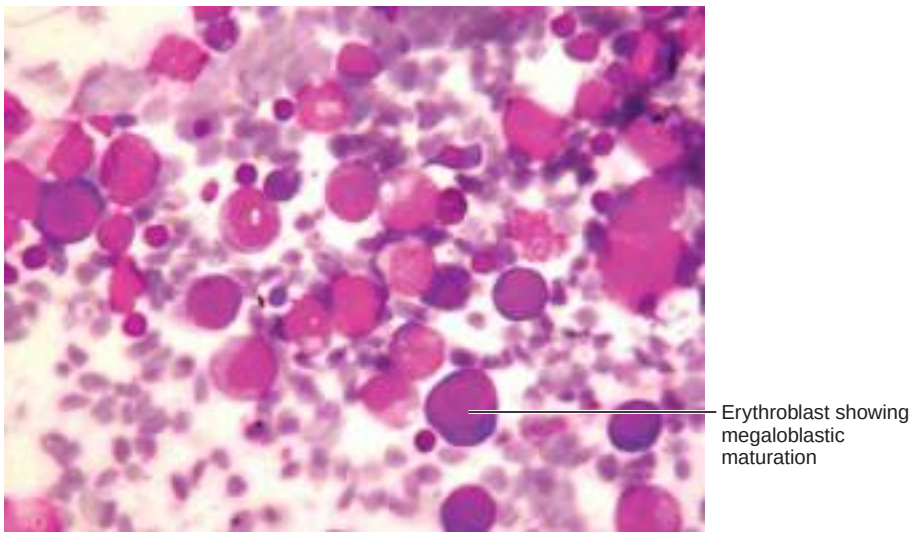


FIGURE 12.2B. Bone marrow aspiration smear showing megaloblastic erythropoiesis.

may also seen in renal failure. MMA levels can be used to monitor the response to treatment. MMA levels remain normal in folate deficiency. **Homocysteine levels** may be elevated with both vitamin B_{12} and folate deficiency. Hyperhomocystinaemia has been linked with increased risk of thrombosis as well as cardiovascular risk.

- Red cells normally contain 20–50 times more folate than serum and red cell folate assay is more reliable than serum folate assay.
- Measurement of urinary excretion of **formiminoglutamic acid (FIGLU)** after giving histidine load was used earlier to assess the folate levels; it is less specific and sensitive than the serum and RBC assays.
- It is necessary to measure folate levels because vitamin B_{12} deficiency must be differentiated from folate deficiency as a cause of megaloblastic anaemia. Folate supplementation can mask vitamin B_{12} deficiency and may improve the anaemia but the neurological deficit continues to progress.
- **Schilling test:** Schilling test is useful for diagnosing intrinsic factor deficiency, as in classic pernicious anaemia. It measures absorption of free radiolabelled vitamin B_{12}. Radiolabelled vitamin B_{12} is given orally, followed in 1–6 h by 1000 mcg (1 mg) of parenteral vitamin B_{12}, which reduces uptake of radiolabelled vitamin B_{12} by the liver. Absorbed radiolabelled vitamin B_{12} is excreted in urine, which is collected for 24 h. The amount excreted is measured and the percentage of radiolabelled vitamin B_{12} is determined. If absorption is normal, $\geq$ 9% of the dose given appears in the urine.

Reduced urinary excretion (if kidney function is normal) indicates inadequate vitamin B_{12} absorption. Improved absorption with the subsequent addition of intrinsic factor to radiolabelled vitamin B_{12} confirms the diagnosis of pernicious anaemia.

The test is often difficult to do or interpret because of incomplete urine collection or renal insufficiency. In addition, because the Schilling test does not measure absorption of protein-bound vitamin B_{12}, the test does not detect defective liberation of vitamin B_{12} from foods, which is common among the elderly.

If the malabsorption is identified, the Schilling test can be repeated after a 2-week trial of an oral antibiotic. If antibiotic therapy corrects malabsorption, the likely cause is intestinal overgrowth of bacteria (eg, blind-loop syndrome).

Q. Write briefly on anaemia due to blood loss.

Ans.

Anaemia due to acute blood loss:

- A healthy adult tolerates a loss of about 500 mL of whole blood without any ill effects.
- When more is lost, compensatory mechanisms come into play (the blood flow to skin and skeletal muscle is reduced, conserving the blood flow to vital organs like brain, kidney and heart.) If bleeding continues, compensatory mechanisms fail and hypovolaemic shock develops.
- Most expansion of plasma volume is seen in the first 24 h of blood loss.

Anaemia due to Chronic Blood Loss

Compensatory mechanisms replenish the plasma volume and red cell loss. However, if the blood loss continues, body iron stores are depleted and anaemia due to iron deficiency appears.

Q. Define normocytic normochromic anaemia and enumerate its causes.

Ans. Normocytic normochromic anaemia is characterized by normal size of RBCs (normal MCV) and normal haemoglobinization (MCH).

Causes of normocytic normochromic anaemia are listed in Table 12.7**.**

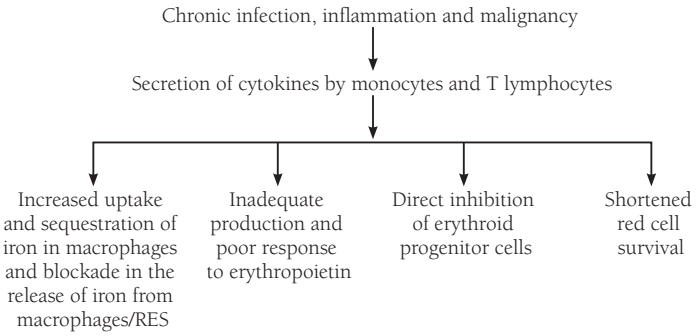
TABLE 12.7. Causes of normocytic normochromic anaemia

Decreased red cell production	Increased red cell loss
• Anaemia of chronic illness	• Acute blood loss
• Marrow hypoplasia or aplasia	• Hypersplenism
• Myeloproliferative diseases	• Haemolytic disorders
• Myelofibrosis	• Haemoglobinopathies (sickle cell disease)
• Chronic renal failure	• Hereditary spherocytosis
• Chronic liver disease	• Glucose-6-phosphate dehydrogenase deficiency
• Sideroblastic anaemia	• Microangiopathic anaemias
• Hypothyroidism	• Autoimmune haemolytic anaemia
• Adrenal insufficiency	• Paroxysmal nocturnal haemoglobinuria

Q. Outline the pathogenesis and laboratory findings of anaemia of chronic disease.

Ans. Anaemia of chronic disease is the most common cause of normocytic anaemia and the second most common form of anaemia worldwide (after the iron-deficiency anaemia). It occurs in a wide variety of chronic diseases including infective or inflammatory conditions, neoplasms and collagen vascular disorders, eg, rheumatoid arthritis. The diagnosis of anaemia of chronic disease is not usually applied to anaemias associated with renal, hepatic or endocrine disorders.

Pathogenesis (Flowchart 12.6)



FLOWCHART 12.6. Pathogenesis of anaemia of chronic disease.

- Restricted movement of iron from reticuloendothelial system (RES) to erythroid cells is due to:
  - Production of **lactoferrin** by inflammatory cells (lactoferrin avidly binds iron; iron bound to lactoferrin is shunted to macrophages as there are no receptors for lactoferrin on erythroid cells)
  - Increased synthesis of **apoferritin** in inflammation (apoferritin binds to increased amounts of iron and diverts circulating iron to storage pool)

Laboratory Investigations

- Peripheral smear shows normocytic normochromic anaemia.
- Serum iron, transferring levels and total iron-binding capacity are reduced.
- Ferritin levels are elevated and reticuloendothelial iron is increased.

Q. Classify haemolytic anaemia.

Ans. Haemolytic anaemias have been classified in Table 12.8.

TABLE 12.8. Classification of haemolytic anaemias

Intrinsic/intracorpuscular abnormalities	Extrinsic/extracorpuscular abnormalities

Hereditary
• Membrane cytoskeleton disorders: Spherocytosis and elliptocytosis
• Red cell enzyme deficiency: Pyruvate kinase, G6PD
• Disorders of haemoglobin synthesis
  • Deficient globin synthesis, eg, thalassaemia syndrome
  • Structural abnormality of globin chain (haemoglobinopathies), eg, sickle cell anaemia
Acquired
• Membrane defect: Paroxysmal nocturnal haemoglobinuria (PNH)
	Immune haemolytic anaemia
	• Autoimmune (idiopathic, SLE, malignant neoplasms)
	• Alloimmune
	• Drug induced
	Mechanical trauma to red cells (microangiopathic haemolytic anaemia)
	Thrombotic thrombocytopenic purpura (TTP), DIC and prosthetic heart valves
	Infections
	Malaria and bacterial diseases
	Chemical injury
	Lead poisoning
	Sequestration in mononuclear phagocyte system
	Hypersplenism

Q. Enumerate the causes of intravascular haemolysis.

Ans. Causes of intravascular haemolysis:

- Microangiopathic haemolytic anaemia (sickle cell anaemia, DIC and TTP)
- Physical injury (mechanical trauma and thermal injury)
- PNH
- G6PD deficiency
- Autoimmune haemolytic anaemia
- Mechanical heart valves
- March haemoglobinuria (seen in vigorous exercise)
- Pregnancy-induced hypertension
- Infections—*P. falciparum* and *Clostridium perfringens*
- Disseminated malignancy
- Haemolytic uraemic syndrome.

Q. Enumerate the causes of extravascular haemolysis.

Ans. Causes of extravascular haemolysis:

- All red cell membrane defects, eg, hereditary spherocytosis
- Sickle cell anaemia
- Premature destruction of RBCs, eg, thalassaemia or other Hb synthesis disorders
- Splenomegaly (hypersplenism)

Q. Enumerate the steps in the laboratory diagnosis of haemolytic anaemia.

Ans. There are three main components of haemolytic anaemia:

1. Premature destruction of red cells
2. Accumulation of products of haemoglobin breakdown
3. Accelerated erythropoiesis in bone marrow

Laboratory Evidence of Increased RBC Breakdown

- Increased serum bilirubin (mainly unconjugated)
- Increased faecal stercobilinogen
- Increased urinary urobilinogen
- Increased plasma LDH (LDH2 > LDH1)

Laboratory Evidence of Intravascular Haemolysis

- Decreased or absent serum haptoglobin and haemopexin (haemoglobin-binding proteins)
- Haemoglobinaemia, haemoglobinuria and methaemalbuminaemia
- Haemosiderinuria
- Jaundice

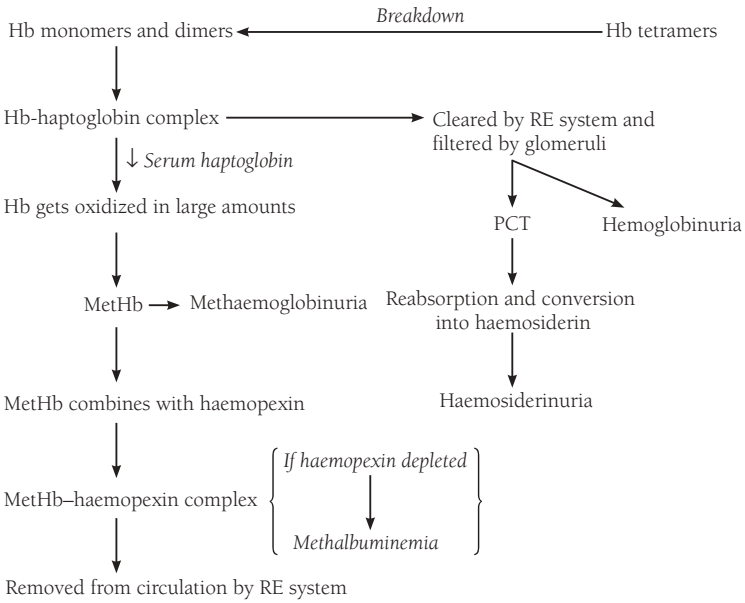
Laboratory Evidence of Compensatory Erythroid Hyperplasia

- Increased reticulocyte count
- Macrocytosis, polychromasia and stippling
- Erythroid hyperplasia

Laboratory Evidence of Damage to RBCs

- Presence of fragments of red cells (schistocytes) and spherocytes in the peripheral smear
- Positive direct Coombs test, if haemolysis is immunological in origin
- Shortened red cell life (decreased to 30–40 days; normal 120 days)

Sequence of Events in Intravascular Haemolysis (Flowchart 12.7)



FLOWCHART 12.7. Sequence of events in intravascular haemolysis.

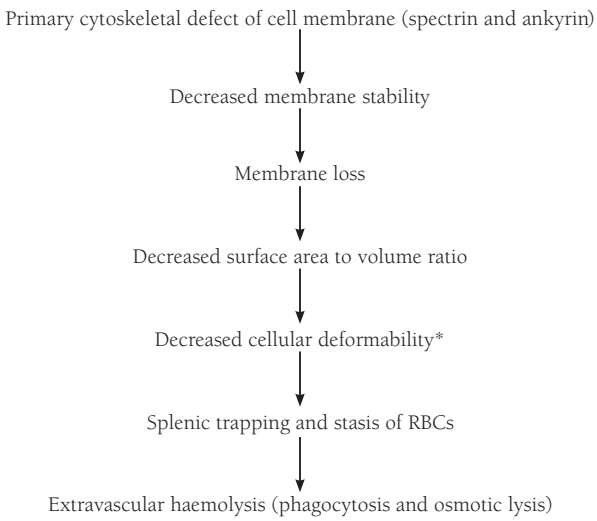
Q. Write briefly on the molecular pathology, laboratory diagnosis and clinical features of hereditary spherocytosis (HS).

Ans. HS is an inherited disease characterized by an intrinsic defect in red cell membrane that results in less deformable, spheroidal RBCs, which are vulnerable to splenic sequestration and destruction. It is autosomal dominant in 75% cases; remaining being recessive.

Molecular Pathology

- Defect in red cell membrane cytoskeleton
- Spectrin, ankyrin, protein 4.1 and band 3 are main cytoskeletal proteins that are responsible for maintenance of normal shape, strength and flexibility of red cell membrane. The most common abnormality is a quantitative reduction in spectrin. In some patients, spectrin is unable to attach to protein 4.1.
- Any defect in these cytoskeleton proteins is associated with reduced membrane stability and loss of membrane fragments as the cells are exposed to shear stress in the circulation.
- Reduction in cell surface to volume ratio forces cells to assume shape of least surface area for a given volume that is a sphere.

Pathophysiology (Flowchart 12.8)



*The discoid shape of the normal RBCs allows extreme degrees of deformation required to leave cords of Billroth and enter the splenic sinusoids. Due to their spherical shape and limited deformability, spherocytes are sequestered in the splenic cords.

FLOWCHART 12.8. Pathophysiology of hereditary spherocytosis.

Clinical Features

- Presents in childhood with anaemia, splenomegaly, jaundice and gallstones
- Crises (**aplastic crisis** triggered by parvovirus B_{19} and **haemolytic crisis**) are commonly encountered.

Laboratory Diagnosis

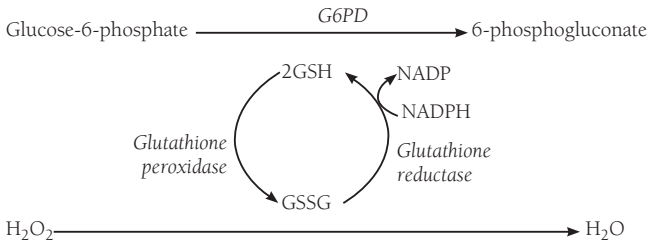
- **General Haematological parameters:**
  - Reduced values of Hb and MCV
  - Increased MCHC
  - Increased reticulocyte count
- **Peripheral smear:** Anisocytosis with presence of microspherocytes and spherocytes (dark appearing red cells with no central pallor). Unlike the spherocytes seen in other conditions, spherocytes in HS are uniform in size and density
- **Bone marrow:** Erythroid hyperplasia (haemolytic picture)
- **Osmotic fragility test:** Determines the susceptibility of RBCs to haemolysis when they are subjected to osmotic stress; osmotic fragility is increased in HS.
- **Autohaemolysis:** Spontaneous haemolysis when blood is incubated at 37°C for 48 h (increased in HS; lysis of 10–15% RBCs as compared to $<$ 4% in normal).
- **Direct Coombs test:** Negative (differentiates it from acquired spherocytosis of AIHA in which Combs test is positive)
- **Increased serum bilirubin**
- **Electrophoretic analysis of spectrin and other cytoskeleton protein levels (confirmatory test)**

Treatment

Splenectomy (after splenectomy, spherocytes persist but anaemia is corrected)

Q. Write briefly on G6PD deficiency anaemia.

Ans. Role of G6PD (glucose-6-phosphate dehydrogenase) enzyme in HMP pathway (Flowchart 12.9):



FLOWCHART 12.9. Role of G6PD (glucose-6-phosphate dehydrogenase) enzyme in HMP pathway.

- Red cells are vulnerable to injury by endogenous and exogenous oxidants, which are normally inactivated by reduced glutathione (GSH).
- Abnormalities that affect enzymes required for GSH production reduce the ability of the cells to protect themselves from oxidative injury, leading to haemolysis.
- Prototype is the haemolytic anaemia associated with deficiency of G6PD.
- More than 400 genetic variants of G6PD have been identified; the mutant gene has an X-linked inheritance.
- **Induction of haemolysis always occurs in the presence of an environmental agent (never spontaneous). Haemolysis develops after a lag period of 2–3 days** and may be:
  - *Drug induced*: Primaquine, chloroquine, sulfonamides, phenacetin and aspirin in large doses
  - *Infection induced*: Viral hepatitis, pneumonia and typhoid fever
  - *Food induced*: Fava beans

Mechanism

- There is production of free radicals as a response to the environmental agents, eg, H_2O_2 which is normally neutralized by GSH. Free radicals induce oxidation of sulphhydryl groups of globin chain.
- Denaturation of Hb chains results in precipitation as **Heinz bodies** (appear as dark inclusions within cells). Attachment of Heinz bodies to membrane aids to deformity of RBCs and intravascular haemolysis.
- When these cells pass through splenic cords, macrophages pluck out Heinz bodies along with cytoplasm giving appearance of **'bite cells'.**
- Loss of membrane results in the formation of spherocytes and extravascular haemolysis.

Laboratory Diagnosis of G6PD Deficiency Anaemia

During Normal Phase

No anaemia is evident but red cell survival is decreased. Defective variant enzymes can be detected by molecular techniques.

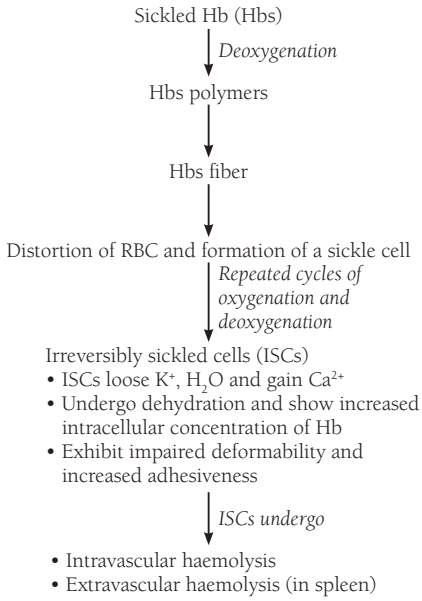
During Haemolytic Phase

- Features of intravascular haemolysis (during active phase)
- Rapid fall of haematocrit with reticulocytosis (during recovery phase)
- *PBS*: Heinz bodies (demonstrated by supravital stains like crystal violet) and bite cells
- *G6PD assay*:
  - Indirect assays, based on decreased ability to reduce dye. Methods used are met haemoglobin reduction test (MRT), fluorescent screening test and ascorbate cyanide screening test.
  - Direct enzyme assay in RBC.

Q. Write briefly on the pathophysiology, clinical features and laboratory diagnosis of sickle cell disease.

Ans. Sickle cell disease is a hereditary haemoglobinopathy, which is characterized by point mutation-substitution of glutamic acid (CTG) by valine (CAG) at 6th position in β-globin chain.

Pathophysiology (Flowchart 12.10)



FLOWCHART 12.10. Pathophysiology of sickle cell anaemia.

Factors Affecting Sickling

- **Amount of HbS:** Heterozygotes do not show sickling except under severe hypoxia.
- **Interaction with other type of Hb:** HbF inhibits polymerization of HbS and hence, sickling (so, the disease manifests 5–6 months after birth). HbC and HbD promote sickling (HbSC is the more severe form of disease).
- **MCHC value:** Any condition (like dehydration) that increases MCHC increases sickling.
- **pH:** Fall in pH increases sickling.
- **Oxygen concentration:** Increased oxygen concentration increases sickling.

Clinical Features

- Severe anaemia and generalized impairment of growth and development due to hypoxia.
- Vasoocclusive complications which include acute chest syndrome, dactylitis (hand-foot syndrome) and stroke.
- Chronic hyperbilirubinaemia and cholelithiasis.
- Septicaemia and meningitis caused by Pneumococci and *H. influenzae* are common. Patients are also predisposed to *Salmonella* osteomyelitis. Increased susceptibility to infection is attributed to
  - Impaired splenic function (autosplenectomy), which occurs as a result of hypoxic tissue damage consequent to chronic stasis and congestion of red pulp.
  - Defect in alternative complement pathway (opsonization defect).
- Other commonly encountered crises include **aplastic crisis** (sudden cessation of marrow erythropoiesis triggered by parvovirus infection manifesting as anaemia without

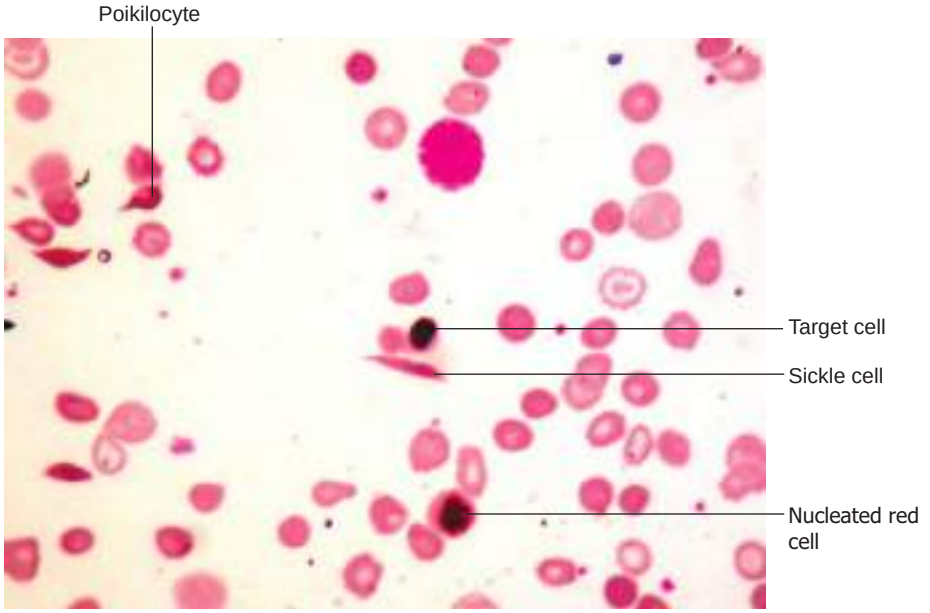


FIGURE 12.3. Red cells show mild hypochromia and anisopoikilocytosis with the presence of microcytes, target cells, sickle cells and poikilocytes.

reticulocytosis) and **splenic sequestration crisis** (sudden pooling of blood in the markedly enlarged spleen which leads to hypovolaemia and shock).
- Tender hepatomegaly (due to infarction)
- Progressive loss of renal function (due to infarction of renal medulla), papillary necrosis and recurrent urinary infections

Laboratory Diagnosis of Sickle Cell Anaemia

Features of both intravascular and extravascular haemolysis are present

- **General blood parameters:** Moderate to severe anaemia (Hb 6–8 g/dL) with reticulocytosis
- **Peripheral blood smear (**Fig. 12.3**):** Presence of sickle cells; Howell–Jolly bodies and nucleated RBCs
- **Sickling test:** This is based on the principle that reducing substances like sodium metabisulphite increase sickling tendency.
- **Solubility test:** This is based on the principle that with reducing substances like sodium dithionite, HbA gives a clear solution; whereas, HbS polymerizes to produce a turbid solution.
- **Hb electrophoresis:**
  - Decreased/absent HbA (normal adult Hb)
  - Increased HbS (abnormal Hb)
  - Increased HbF (2–20%, compensatory increase)
- **Osmotic fragility test:** Osmotic fragility is decreased due to the sickle shape which has large scope for expansion of volume without rupture of the red cell.

Q. Describe the molecular pathology, clinical features and laboratory diagnosis of thalassaemias.

Ans. Normally, HbA is the predominant type of haemoglobin found in adults. It comprises two α chains and two β chains; β chains are coded by two globin genes, each located on one of the two chromosome 11; whereas, α chains are coded by two pairs of genes, one pair located on each chromosome 16. Thalassaemias are a heterogenous group of genetic disorders characterized by a reduction in the synthesis of one or more haemoglobin polypeptide chains.

Common Types of Thalassaemias

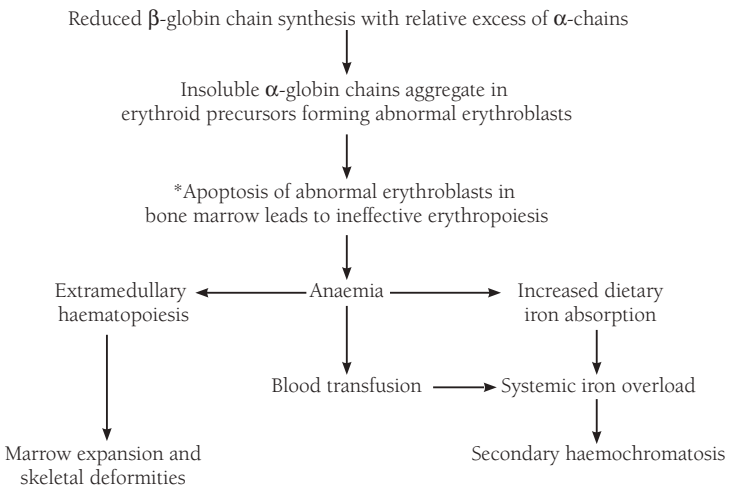
1. **α-Thalassaemia:** Affects the synthesis of α chains and its severity depends on the number of α genes deleted. Based on the number of dysfunctional α genes, α-thalassaemia is classified into
   (a) **α-thalassaemia trait:** One or two α genes are deleted or dysfunctional. Majority of the patients are asymptomatic; some show reduced MCV and MCH and a microcytic hypochromic anaemia.
   (b) Haemoglobin H disease: This is caused by functional inactivation of 3–4 α genes. There is microcytic hypochromic anaemia (Hb-7–11 g/dL); hepatosplenomegaly, jaundice, gall stones and leg ulcers. Haemoglobin electrophoresis shows 4–10% HbH (β4 haemoglobin). No bony deformities or features of iron overload are evident.
   (c) Haemoglobin Bart's (hydrops fetalis): All four α genes are inactive. There is inability to make either HbA or HbF. The excess β chains form Hb Bart's. There is intrauterine death at 25–40 weeks or the fetus dies immediately after birth.
2. **β-Thalassaemia:** Also called *Cooley anaemia or Mediterranean anaemia,* β-thalassaemia is characterized by a total lack or reduction in the synthesis of structurally normal β-globin chains with normal synthesis of α chains resulting in reduced levels of HbA. β-thalassaemia is a common blood disorder, which occurs most frequently in Mediterranean countries, North Africa, the Middle East, India, Central and Southeast Asia. Depending on the severity it is classified into
   (a) β-Thalassaemia minor (trait)
   (b) β-Thalassaemia intermedia
   (c) β-Thalassaemia major

Molecular Pathology of β-Thalassaemia

Based on molecular pathology β-thalassaemia is classified into

- β^0-T**halassaemia:** Total absence of β chains (homozygous state)
- β^+-**Thalassaemia:** Reduced synthesis of β chains (homozygous state)
- β-**Thalassaemia** is mainly due to point mutations (in contrast to gene deletion in α-thalassaemia).
- **Promoter region mutations** lead to β^+-thalassaemia.
- **Chain terminator mutations** lead to β^0-thalassaemia. These result from either of the two following mechanisms:
  - Frame shift mutation (introduction of stop codon)
  - Point mutation (introduction of stop codon)
- **Splicing mutations** (most common cause of thalassaemia) may occur:
  - *At the junction of exon and intron:* β^0-thalassaemia
  - *In intron:* β^+-thalassaemia
- **Translation defect of exon** leads to β^0-thalassaemia.
- β-***Thalassaemia major may be***
  - Homozygous β^0-thalassaemia (β^0/β^0)
  - Homozygous β^+-thalassaemia (β^+/β^+)
  - Double heterozygous (β^+/β^0) thalassaemia.
- β-***Thalassaemia minor/trait is***
  - Heterozygous (β^0/β, β^+/β)

Pathogenesis (Flowchart 12.11)



*Few abnormal erythroblasts with insoluble α-globin aggregates leave bone marrow and undergo extravascular haemolysis in spleen.

FLOWCHART 12.11. Pathogenesis of β-thalassaemia.

Clinical Features of β-Thalassaemia

- **β-Thalassaemia major**
  - Manifests 6–9 months after birth (as HbF decreases)
  - Presents with severe anaemia, requiring regular blood transfusions
  - Untransfused patients show failure to thrive; growth retardation and early death
  - **Mongoloid** or **thalassaemia facies** is typical (marrow expands due to erythroid hyperplasia leading to bossing of skull, hypertrophied maxillae and hair on end appearance on X-ray).
  - Extramedullary haematopoiesis may lead to hepatosplenomegaly. Other manifestations include recurrent infections, spontaneous fractures, hypersplenism and leg ulcers.
  - Transfused patients may end up with secondary haemochromatosis (iron chelators are given for treatment of the same). Iron deposition in the pancreas, liver and heart leads to diabetes, cirrhosis and arryhtmias, heart block or cardiac failure, respectively.
  - ***Definite prevention and treatment of* β-thalassaemia major:**
    1. Prenatal diagnosis by DNA analysis and abortion
    2. Bone marrow transplantation from HLA-identical sibling
- **β-Thalassaemia minor (trait)**

Patient is asymptomatic with mild or no anaemia and is commonly is diagnosed accidentally on peripheral smear examination.

Laboratory Diagnosis of β-Thalassaemia Major

General Blood Parameters

- Hb varies between 2 and 6 g/dL.
- Haematocrit, MCV, MCH and MCHC are severely decreased.
- RBC count is decreased.
- WBC count is increased with a shift to left of neutrophil series (presence of myelocytes and metamyelocytes).
- Platelet count may be normal or decreased (decrease is due to massive splenomegaly).

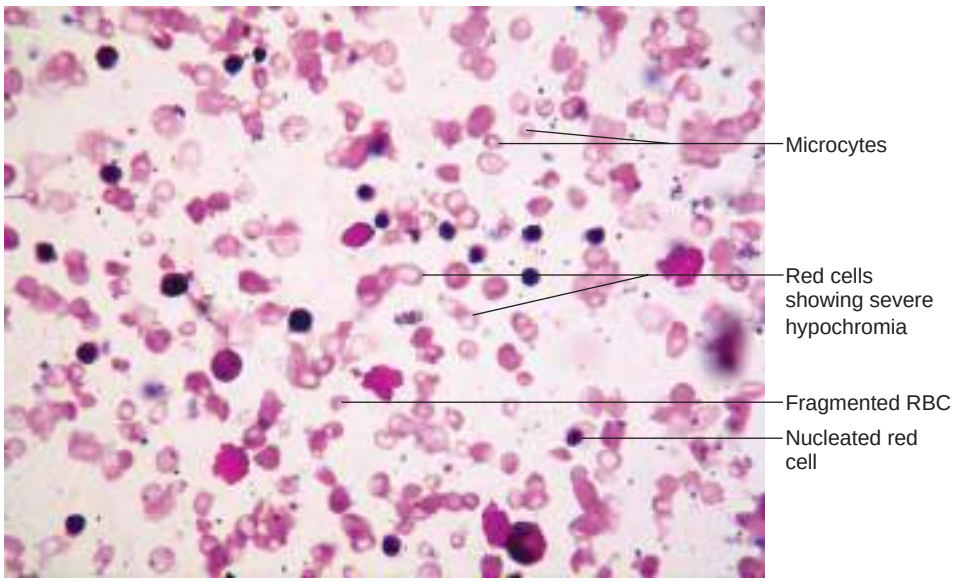


FIGURE 12.4. Leishman-stained PBS of β-thalassaemia major showing severe microcytic hypochromic anaemia with marked anisopoikilocytosis. There is presence of target cells, teardrop cells, fragmented red cells and nucleated red cells.

Peripheral Blood Smear (Fig. 12.4)

- Severe microcytic hypochromic anaemia with marked anisopoikilocytosis
- Basophilic stippling, target cells, poikilocytes, fragmented red cells, pencil cells, cells with Cabot rings and numerous nucleated red cells.

Bone Marrow

- Normoblastic erythroid hyperplasia
- Ineffective erythropoiesis
- Predominance of intermediate and late normoblasts (smaller in size than normal)
- Increased reticuloendothelial iron with siderotic granules in the cytoplasm of normoblasts

Hb Electrophoresis

- HbA: Absent/markedly decreased
- HbA_2: Normal/decreased/increased
- HbF: Markedly increased (10–98%)

Other Findings

- Increased unconjugated bilirubin and urinary urobilinogen
- Markedly increased serum iron
- Increased percentage transferrin saturation (> 70%)
- Markedly increased serum ferritin (300–3000 mg/dL)
- Decreased osmotic fragility

Laboratory Diagnosis of β-Thalassaemia Trait

General Blood Parameters

- Hb and haematocrit mildly decreased with increase in the reticulocyte count.
- MCV, MCH and MCHC decreased out of proportion to degree of anaemia.
- RBC count higher as compared to iron deficiency anaemia for the same haemoglobin value.

Peripheral Blood Smear

- Hypochromic microcytic RBCs with mild anisopoikilocytosis
- Target cells, basophilic stippling, poikilocytes, pencil cells, cells with Cabot rings and nucleated red cells may be present but are fewer as compared to β-thalassaemia major.

Bone Marrow

Mild erythroid hyperplasia.

Haemoglobin Electrophoresis

HbA_2 increased (3.6–9%; normal 1–3.5%)

Osmotic Fragility Test

Shows decreased osmotic fragility

Q. Write briefly on paroxysmal nocturnal haemoglobinuria (PNH).

Ans. PNH is the only example of an acquired defect in red cell membrane. It is characterized by chronic haemolytic anaemia with intermittent haemoglobinuria. It may be associated with aplastic anaemia, myelodysplastic syndrome and rarely acute leukaemia.

Pathology

- Mutation in phosphatidylinositol glycan A (PIGA) gene that codes for glycosyl-phosphatidylinositol (GPI) protein, which acts as an anchor of GPI-linked proteins to the cell membrane.
- GPI-linked membrane proteins regulate complement factors and are absent in PNH. These are
  - CD55 or decay-accelerating factor
  - CD59 or membrane inhibitor of reactive lysis
  - C8-binding protein (homologous restriction factor)

GPI-linked proteins interact with C3b and C4b to dissociate the convertase complexes of both classic and alternative complement pathways thus stopping amplification of activation by complement. RBCs, platelets and granulocytes are more sensitive to complement lysis when these proteins are absent.

Laboratory Diagnosis

General Blood Parameters

- **Evidence** of intravascular haemolysis
- **Decreased** Hb, RBC, WBC and platelet counts (pancytopenia)
- **Increased** reticulocyte count and occasionally raised HbF

Peripheral Blood Smear

Anaemia with macrocytosis and polychromasia

Bone Marrow

- Hypercellular marrow with normoblastic erythroid hyperplasia
- Some dyserythropoiesis is seen.
- Iron stores are decreased.
- Intermittent clinical haemoglobinuria (acute haemolytic episodes which occur mostly at night and are identified by passage of brown coloured urine in the morning).
- Haemosiderinuria and venous thrombosis are common.

Sucrose Haemolysis Test

A screening test for PNH, it is more sensitive than Hams test given below, though lacks specificity. Sucrose enhances complement binding to RBCs and haemolysis is by classic pathway of complement. Sucrose lysis test is done to find out degree of haemolysis ($>$ 10% haemolysis is diagnostic of PNH).

Hams Test (for Definitive Diagnosis of PNH)

The patient's cells undergo haemolysis (by alternative complement pathway) in compatible acidified serum at 37°C. The serum may be the patient's own or from another normal subject. Ten to fifty percent lysis indicates a positive test.

Q. Write briefly on autoimmune acquired haemolytic anaemia (AIHA).

Ans. AIHAs are a group of acquired disorders in which antibodies develop against red cell antigens and cause destruction of red cells.

Classification

1. **Based on antibody type**
   (a) Warm antibody AIHA
      (i) Primary or idiopathic
      (ii) Secondary
         - Drugs (methyldopa, penicillin and quinidine)
         - Autoimmune disorders (SLE, others)
         - Haematologic malignancies like chronic lymphocytic leukaemia and lymphomas
   (b) Cold antibody AIHA
      (i) Cold haemagglutinin disease
      (ii) Paroxysmal cold haemoglobinuria
      (iii) Cold AIHA associated with mycoplasma infection
2. **Based on aetiology**
   (a) Idiopathic autoimmune acquired haemolytic anaemia (50%)
   (b) Secondary autoimmune acquired haemolytic anaemia (50%)
      (i) Drugs, eg, methyldopa, penicillins, procainamide and phenothiazine
      (ii) Chronic lymphocytic leukaemia
      (iii) Malignant disorders like lymphomas
      (iv) Infections like *M. pneumoniae*, infectious mononucleosis, cytomegalovirus and rubella
      (iv) SLE and other connective tissue disorders
      (v) Immune deficiency states (common variable immunodeficiency)
      (vi) Miscellaneous: Carcinoma, sarcoidosis, ovarian teratoma posttransplant

Warm Antibody AIHA

- Most common form of immune haemolytic anaemia
- Caused by warm antibodies, which react with RBCs at 37°C
- Majority of warm antibodies are of the IgG class
- Most RBC destruction is extravascular. IgG-coated RBCs bind to Fc receptors on macrophages resulting in loss of RBC membrane during passage through spleen. This converts the RBCs to spherocytes, which are removed by the spleen.
- Clinical features include anaemia, jaundice, hepatosplenomegaly and manifestations of underlying disease.
- Diagnosis is based on presence of anaemia with reticulocytosis, evidence of haemolysis, spherocytes in peripheral blood and positive direct (antibodies on the red cell surface) and indirect Coombs tests (antibodies in the serum).

Cold Antibody Autoimmune Haemolytic Anaemia

- Caused by cold agglutinins, which are IgM antibodies that bind and agglutinate RBCs at low temperatures (0–4°C)
- It is of two types, cold haemagglutinin disease (CHAD) and paroxysmal cold haemoglobinuria (PCH). CHAD is characterized by a haemolytic anaemia due to autoantibodies that act as RBC agglutinins at low temperatures; whereas, PCH is characterized by episodes of acute haemolysis due to autoantibodies that act as red cell lysins at low temperatures.
- Most cells with bound IgM pick up C3b but are not lysed in the periphery.
- When they travel to warmer areas, the weakly bound IgM is released, but the coated C3b remains.

- C3b being an opsonin, the cells are phagocytosed by the mononuclear phagocytic system (haemolysis is extravascular).
- The typical blood picture is of anaemia with reticulocytosis, red cell agglutination and a positive direct antiglobulin test.

Q. Write briefly on Coombs test.

Ans. Coombs test can be

1. Direct Coombs Test

In this test, patient's red cells are washed and suspended in saline. Rabbit anti-human globulin is added. Agglutination of red cells indicates the presence of antibodies on the surface of red cells.

***Indications*:**
- Haemolytic disease of newborn
- Autoimmune haemolytic anaemia

2. Indirect Coombs Test

- In this test, normal red cells and rabbit anti-human globulin are added to the patient's serum. This produces agglutination of red cells if antibodies are present in the serum.
- This detects the incomplete antibodies present in a person's serum.
- The serum from the patient is taken and added to a suspension of O^+ RBCs. If the serum contains incomplete antibodies against Rh antigen, it will coat the O^+ RBCs. The suspension is washed many times to remove excess unbound antibodies in the serum.
- Thereafter, Coombs serum is added. If agglutination occurs, the test is said to be positive.

***Indications*:**
- In crossmatching of blood to detect incomplete antibodies in donor's serum
- In case of Rh-negative mother, whose first child is Rh-positive, and wants second conception

Q. Define pancytopenia. Enumerate its causes.

Ans. Pancytopenia is defined as simultaneous presence of anaemia (Hb $<$ 13.5 g/dL), leucopenia (TLC$<$ 4 $\times$ 10^9/L) and thrombocytopenia (150 $\times$ 10^9/L).

Causes
- **Hypocellular bone marrow:** Aplastic anaemia, hypoplastic MDS, cytotoxic drugs and radiotherapy
- **Cellular marrow with systemic disease:** Megaloblastic anaemia, hypersplenism, tuberculosis, Kala-azar, brucellosis, severe infection, alcohol and autoimmune diseases
- **Cellular marrow with primary marrow disease:** Bone marrow infiltration as seen in lymphoma, acute leukaemia, myeloma, carcinoma, paroxysmal nocturnal haemoglobinuria, disseminated tuberculosis, myelofibrosis and marrow metastasis

Q. Write briefly on aplastic anaemia.

Ans. Aplastic anaemia is a condition in which bone marrow failure results in pancytopenia (anaemia, granulocytopenia and thrombocytopenia) in the absence of any abnormal cells in marrow or blood.

Classification of Aplastic Anaemia

1. **Congenital:** Fanconi's anaemia and Schwachman–Diamond syndrome
2. **Acquired:** Primary or idiopathic (no definite cause) and secondary (definite or likely agent can be identified)

Causes of Secondary or Acquired Aplastic Anaemia

- **Infections:** Hepatitis viruses, EBV, human immunodeficiency virus (HIV), parvovirus and mycobacteria
- **Radiation and chemicals:** Benzene, lindane (gamma benzene hexachloride) and DDT
- **Drugs:** Drugs can produce aplastic anaemia either due to direct toxic effect (dose-dependent and predictable response) or idiosyncratic reactions (dose-independent and unpredictable response). The following drugs are implicated in aplastic anaemia:
  - Cytotoxic (alkylating agents and antimetabolites)
  - Antibacterial (chloramphenicol, sulfonamides, isoniazid and arsenicals)
  - Antirheumatic (oxyphenbutazone, phenylbutazone, indomethacin, gold salts and D-penicillamine)
  - Antidiabetic (tolbutamide and chlorpropamide)
  - Miscellaneous (chlorothiazide, mepacrine, hydralazine, acetazolamide, potassium perchlorate, carbamazepine and carbimazole)
- **Miscellaneous causes:** Pancreatitis, PNH and eosinophilic fasciitis

Pathogenesis

Haematopoietic failure may be due to various mechanisms, eg, decreased number of stem cells in the marrow, defective stem cells or a defective microenvironment that fails to sustain normal haematopoiesis.

Clinical Features

- Petechiae, ecchymoses, nasal and GIT bleeding due to thrombocytopenia
- Infections due to neutropenia
- Weakness, easy fatigability, pallor and breathlessness due to anaemia

Laboratory Diagnosis

Aplastic anaemia is diagnosed if any two of the following are present:

- Hb $\leq$ 10 g/dL
- Neutrophil count $\leq$1500/mm^3
- Platelet count $\leq$50,000/mm^3

Peripheral Smear

- Shows a normocytic-normochromic anaemia, leucopenia (neutropenia with relative lymphocytosis) and thrombocytopenia
- Mild macrocytosis is occasionally seen.
- Corrected reticulocyte count is low.
- May be differentiated from **infiltrative causes of pancytopenia** based on the absence of teardrop poikilocytes and a leukoerythroblastic picture, both of which suggest an infiltrative process. The presence of dyserythropoietic cells and hypogranulated neutrophils indicates **myelodysplasia** and differentiates aplastic anemia from dysplastic causes of pancytopenia.

Bone Marrow

Dry tap; markedly hypocellular or acellular marrow with increased iron stores

Grading of Aplastic Anaemia

Aplasia is said to be 'severe' if any two of the following are present:

1. Neutrophil count is less than 500/mm^3.
2. Platelet count is less than 20,000/mm^3.
3. Absolute reticulocyte count $<$40,000/mm^3 and marrow biopsy showing $<$25% of normal cellularity, or 25–50% marrow cellularity with $<$30% haematopoietic cells.

Criteria for 'very severe' aplasia are similar, except granulocyte count $\leq$ 200/mm^3.

Q. Enumerate the disorders of WBCs.

Ans. The main disorders of WBCs are: Leukocytosis: Increase in the number of circulating leukocytes beyond the upper limit of normal (> 11,000/mm^3; normal range 4000–11,000/mm^3)
Leucopenia: Total leukocyte count below the lower limit of normal (< 4000/mm^3)
Leukoerythroblastic reaction: Presence of immature WBCs as well as nucleated red cells in the peripheral blood
Leukaemoid reaction: Markedly increased leukocyte count with the presence of immature white cells in the peripheral blood but nonleukaemic in origin

Q. Write briefly on the quantitative disorders of neutrophils.

Ans. Neutrophilia is defined as absolute peripheral neutrophil count more than 7500/mm^3.

Causes of Neutrophilia:

1. **Acute infections: Furuncles, abscesses, tonsillitis, appendicitis, otitis media, osteomyelitis, cholecystitis, salpingitis, meningitis and peritonitis caused by** Gram-positive cocci, (eg, staphylococci, streptococci, pneumococci, meningococci and gonococci), *Escherichia coli, Pseudomonas aeruginosa, Actinomycosis,* certain fungi (eg, *Coccidioides immitis*), spirochetes and viruses (rabies, poliomyelitis, herpes zoster and varicella), rickettsiae and parasites.
2. **Noninfectious causes:** Burns, postoperative state, acute myocardial infarction, acute attacks of gout, acute glomerulonephritis, rheumatic fever and collagen vascular diseases, Hodgkin lymphoma and solid tumours.
   Neutrophilia may be accompanied by a shift to the left and the presence of **toxic granules** and **Döhle bodies.**
   (a) **Toxic granules:** Dark blue/purple granules in the cytoplasm of neutrophils. They represent azurophilic granules and result from impaired cytoplasmic maturation during accelerated generation of neutrophils.
   (b) **Döhle bodies:** Pale inclusion bodies in the periphery of cytoplasm of neutrophils, which represent rough endoplasmic reticulum.

Neutropenia is defined as a reduction in the number of neutrophils to less than 2000/mm^3. Its causes include drugs (antimicrobials, analgesics and cytotoxic drugs), infections (septicaemia, military tuberculosis, HIV, influenza and infectious mononucleosis), immune neutropenia (Felty syndrome, SLE and neonatal isoimmune neutropenia), megaloblastic anaemia, hypersplenism, aplastic anaemia and bone marrow replacement (leukaemias, myeloproliferative disorders, MDS, myeloma and lymphoma).

Q. Write briefly on quantitative disorders of eosinophils.

Ans. Eosinophilia is defined as the absolute eosinophil count exceeding 600/mm^3.

Causes of Eosinophilia

- **Parasitic infestations:** Ascariasis, toxocara, filariasis, strongyloidosis and trichinosis
- **Pulmonary disorders:**
  - *Loeffler syndrome*: Transient lung infiltrates on X-ray chest, eosinophilia and cough caused due to migration of helminthic larva through the lungs
  - *Tropical pulmonary eosinophilia*: Seen in filaria endemic regions; characterized by cough with wheezing, lung infiltrates and eosinophilia
- **Type I hypersensitivity reactions:** Hay fever, asthma, urticaria and rhinitis
- **Malignancies:** Hodgkin disease, chronic myeloid leukaemia and eosinophilic leukaemia
- **Drugs:** Penicillin and iodides
- **Idiopathic hypereosinophilic syndrome** (persistent high eosinophilia > 1500/mm^3 for more than 6 months without any identifiable cause and with the evidence of organ involvement and dysfunction due to cytokines released from eosinophilic granules)
- **Collagen vascular diseases:** Rheumatoid arthritis and Churg–Strauss syndrome
- **Skin diseases:** Atopic dermatitis, Bullous pemphigoid and eczema

Eosinopenia is caused by steroid administration, acute stress and Cushing syndrome.

Q. Enumerate the causes of monocytosis.

Ans. Monocytosis is defined as a peripheral monocyte count more than 1000/mm^3.

Causes of Monocytosis

- **Infections:** Tuberculosis, brucellosis, listeriosis, bacterial endocarditis, syphilis, infectious mononucleosis and other viral infections, protozoal and rickettsial infections (eg, kala-azar, malaria and Rocky Mountain spotted fever)
- **Autoimmune diseases:** Systemic lupus erythaematosus, rheumatoid arthritis and inflammatory bowel disease
- **Malignancies:** Hodgkin disease, MDS and certain leukaemias, such as chronic myelomonocytic leukaemia (CMML) and AML-M4 and AML-M5
- **Miscellaneous:** Sarcoidosis and carcinomas

Q. Enumerate the causes of basophilia.

Ans. Basophilia is defined as increase in basophil count to more than 100/mm^3.

Causes of Basophilia

- **Inflammatory conditions:** Inflammatory bowel disease, chronic airway inflammation, chronic dermatitis, viral infections and chronic sinusitis
- **Myeloproliferative disorders:** Chronic myelogenous leukaemia, polycythaemia vera and myelofibrosis
- **Endocrinological causes:** Hypothyroidism, ovulation and oestrogens
- **Others:** Chronic haemolytic anaemia, Hodgkin disease and splenectomy

Q. Write briefly on quantitative disorders of lymphocytes.

Ans. Absolute lymphocytosis is defined as increase in the absolute count of lymphocytes beyond 4000/mm^3 in adults.

Causes of Lymphocytosis

- **Infections** like pertussis, infectious mononucleosis, brucellosis, tuberculosis, secondary syphilis, cytomegalovirus, EBV, mumps, measles, varicella, toxoplasmosis and infective hepatitis
- **Malignancies** like ALL, CLL and NHL
- **Autoimmune disorders** like SLE
- **Drugs** like phenytoin

Lymphopenia is caused by aplastic anaemia, high dose of steroids, AIDS, Hodgkin lymphoma and irradiation.

Q. What are leukaemoid reactions?

Ans. Leukaemoid reactions are characterized by an increase in the total leukocyte count beyond 25,000/μL. They are seen in response to infections, haematological and nonhaematological malignancies and various toxic states. The bone marrow shows proliferation without presence of any abnormal cells. Leukemoid reactions are of two types:

1. **Myeloid leukaemoid reactions**
   Total WBC count is markedly increased with a predominance of cells of myeloid series including an occasional immature cell (myelocytes, promyelocytes and myeloblasts).
   Causes:
   - Infections like pneumonia, septicaemia and meningococcal meningitis
   - Secondary to nonhaematological malignancies
   - Acute haemolysis
   - Eclampsia
   - Severe burns

2. **Lymphoid leukemoid reactions**
   - Infections like infectious mononucleosis, cytomegalovirus, pertussis, mumps, measles, rubella, tuberculosis, syphilis, brucellosis and infective hepatitis
   - CLL
   - Carcinoma

Q. Differentiate between leukemoid reactions and chromic myeloid leukaemia (CML).

Ans. Comparative features of leukemoid reactions and CML are tabulated in Table 12.9.

TABLE 12.9. Comparison between leukaemoid reactions and CML

Features	Leukaemoid reaction	CML
Clinical features	Clinical features of the causative disorder	Splenomegaly, lymph node enlargement and anaemia
Blood examination		
• TLC	Moderate increase; rarely exceeds 100×10^9/L	Usual range 20–500 $\times 10^9$/L
• Immature cells	Usually few	Usually numerous
• WBC morphology	**Toxic granules** (increased number of intensely staining primary granules) and **Dohle bodies** (discrete round to oval cytoplasmic bodies, 1–2 microns, stain blue-grey with Romanowski stains) seen in infective cases	Uncommon
• Eosinophilia and basophilia	Absolute eosinophilia or basophilia not seen	Absolute eosinophilia or basophilia seen
• Anaemia	Slight or absent	Present and progressive
• Platelets	Normal or increased	Increased; may decrease in accelerated phase and blast crisis
• Leukocyte alkaline phosphatase (LAP) score	High	Low
Autopsy	No infiltration of organs and tissues	Leukaemic infiltration of organs and tissues is present

Q. Define and enumerate the myeloproliferative disorders?

Ans. Myeloproliferative disorders occur due to clonal expansion of a multipotent haematopoietic progenitor cells with the overproduction of one or more of the formed elements of the blood. These conditions may evolve into acute leukaemia. The following conditions are included under this category of diseases:

- CML
- Polycythaemia vera
- Essential thrombocythaemia
- Primary myelofibrosis
- Systemic mastocytosis
- Chronic eosinophilic leukaemia
- Stem cell leukaemia

Q. Define and classify polycythaemia?

Ans. It is defined as neoplastic proliferation of erythroid, granulocytic and megakaryocytic elements. Polycythaemia can be classified as:

1. **Relative:** Relative polycythemia results from haemoconcentration due to reduced plasma volume (seen in dehydration—low fluid intake, vomiting, diaorrhea and excessive sweating). The red cell mass remains within the normal range in relative polycythemia.

2. **Absolute:** Absolute polycythaemia is associated with an actual increase in the red cell mass and is of two further types:
   (a) **Primary (polycythaemia vera):** Denotes absolute polycythaemia of unknown aetiology, which is associated with decreased erythropoietin levels.
   (b) **Secondary (erythrocytosis):** Erythrocytosis secondary to increased production of erythropoietin as a consequence of hypoxia. It is seen in association with the following conditions:
      (i) High altitude
      (ii) Cyanotic congenital heart diseases (TOF—Tetralogy of Fallot and Eisenmenger complex)
      (iii) Pulmonary diseases (eg, COPD)
      (iv) Chronic carbon monoxide poisoning and smoking
      (v) Abnormal haemoglobin with high oxygen affinity
      (vi) Increased production of erythropoietin or erythropoietin-like substance by tumours and other conditions, as in, cerebellar haemangioblastoma, renal tumours (carcinoma, adenoma and sarcoma), polycystic kidney disease, uterine leiomyoma, hepatocellular carcinoma and pheochromocytoma

Q. Outline the clinical features and laboratory diagnosis of polycythaemia vera.

Ans. Polycythaemia vera is a clonal stem cell disorder characterized by an increased production of formed elements of blood by a hyperplastic marrow; however, the disease is generally dominated by an elevated haemoglobin concentration (haematocrit $>$ 52% in an adult male and $>$ 48% in an adult female).

Aetiology

Unknown; mutation in JAK2, a tyrosine kinase involved in signalling pathway of the erythropoietin receptor, is thought to render the erythropoietin receptor hypersensitive to erythropoietin.

Clinical Features

Seen in middle-aged males who present with dusky red colour of the face (ruddy cyanosis). Complaints are related to the increased viscosity and stasis of blood and include

- Headache, dizziness, vertigo, visual disturbances, tinnitus and syncope (due to decreased cerebral perfusion)
- Pruritus (due to histamine release from neoplastic basophils and mast cells)
- Peptic ulceration (due to excessive histamine)
- Splenomegaly and hepatomegaly
- Symptoms of peripheral vascular insufficiency and thrombotic complications usually affecting the brain and heart; hepatic vein thrombosis resulting in Budd–Chiari syndrome (due to stasis)
- Bleeding manifestations like epistaxis, bleeding from peptic ulcer, intramuscular haemorrhages and bruising (due to platelet function abnormalities).
- Hyperuricaemia (due to rapid cell turn over) may result in the formation of urate stones and nephropathy.

Laboratory Diagnosis

- Markedly elevated haemoglobin concentration and haematocrit (Hb is in the range of 18–24 g/dL and PCV ranges between 0.60 and 0.70)
- Increased red cell mass and blood viscosity
- Total white cell count and platelet count are elevated; absolute basophil count is increased.
- The arterial oxygen saturation is normal in contrast to hypoxic erythrocytosis where it is reduced.
- Bone marrow shows either erythroid hyperplasia or pan hyperplasia.
- Iron stores are depleted.
- Urine and serum levels of erythropoietin are reduced.

Q. Differentiate between primary and secondary polycythaemia.

Ans. Contrasting features of primary and secondary polycythaemia are tabulated in Table 12.10.

TABLE 12.10. Comparison between primary and secondary polycythaemia

Features	Primary polycythaemia	Secondary polycythaemia
Aetiology	Neoplastic disorder	Caused by hypoxia
Facies	Brick red	Cyanosed
Pruritus	Common	Absent
Oxygen saturation	Normal	Low
Erythropoietin levels	Decreased	Increased
Total white cell count	Increased	Normal
Absolute basophil count	Increased	Normal
Platelet count	Increased	Normal
Leukocyte alkaline Phosphatase	Increased	Normal
Vitamin B_{12} levels	Increased	Normal
Bone marrow	Panhyperplasia	Erythroid hyperplasia
Splenomegaly	Present	Absent

Q. Classify leukaemias?

Ans. ***Classification of leukaemias***

1. **FAB (French–American–British) classification:**
   (a) Acute leukaemias
   - Myeloid (myeloblastic); Table 12.11
   - Lymphoid (lymphoblastic); Table 12.12

TABLE 12.11. FAB classification of acute myeloid leukaemias (AML)

M0	Minimally differentiated	Undifferentiated by light microscopy, however myeloid nature is evident on electron microscopy or immunological cell marker studies (presence of one or more myeloid antigens like CD13, CD33 and CD117). B- and T-lymphoid markers are absent. Immunophenotyping is essential for differentiating from ALL
M1	Myeloblastic leukaemia without maturation	Minimal maturation; some blast cell show few granules. Cytochemically >/= 3% blasts are peroxidase-positive. Immunological cell marker studies reveal expression of at least two myeloid antigens (CD13, CD33, CD117or MPO)
M2	Myeloblastic leukaemia with maturation	Most frequent subtype. Auer rods (aggregates of azurophilic granules in lysosomes) are commonly seen. There is clear evidence of maturation to promyelocyte stage and beyond. Blasts constitute between 20 and 89% of the nucleated cells in the marrow. Mature cells (promyelocytes to granulocytes) are > 10%. Monocytic cells should be less than 20%
M3	• Hypergranular promyelocytic leukaemia	Predominance of abnormal promyelocytes, which are hypergranular and show innumerable large azurophilic granules in the cytoplasm. Auer rods are arranged in bundles called **faggots**. Pancytopenia is typical. Myeloperoxidase is strongly positive. There is formation of a fusion gene RARα-PML due to t(15;17) that arrests the maturation of myeloid cells at the promyelocytic stage
	• Microgranular variant	Marked leukocytosis with hypogranular promyelocytes having a typical bilobed nucleus
M4	• Myelomonocytic leukaemia	Blasts are > 20% of the nucleated cells in the marrow. Monocytic cells and their precursors and neutrophils and their precursors are each more than 20%. Nonspecific esterase is positive in cells of monocytic lineage. Myeloperoxidase is positive in more than 3% blasts. Leukaemic cells express myeloid-associated antigens (CD13 and CD33) and markers of monocytic differentiation (CD14 and lysozyme)

Continued

TABLE 12.11. FAB classification of acute myeloid leukaemias (AML)—cont'd

	• M4Eo Variant	Shows increase in marrow eosinophils
M5	Monocytic leukaemia	More than 80% cells in the bone marrow are monocytic (monoblasts, promonocytes and monocytes)
	(a) Undifferentiated (monoblastic) M5a	In M5a, 80% or more cells are monoblasts
	(b) Well-differentiated (promonocytic-monocytic) M5b	In M5b, predominant cells are promonocytes and monocytes
		Variable expression of myeloid antigens CD33, CD13 and CD117
		Monocytic markers CD14, CD36, CD64 and CD11c are positive
M6	Erythroleukaemia (Di Guglielmo disease)	Predominance of erythroblasts. It has two subtypes: **Erythroleukaemia** (> 20% of nonerythroid cells are myeloblasts and > 50% of all nucleated cells are erythroblasts) and **pure erythroid leukaemia** (> 80% of marrow cells are erythroblasts). Erythroblasts may be bizarre looking with bi- and trinucleate forms and megaloblastic nuclear features and are positive for monoclonal antibody against glycophorin A
M7	Megakaryoblastic leukaemia	Blasts are more than 20% of which at least 50% are of megakaryocytic origin. Megakaryoblasts resemble lymphoblasts but show distinct cytoplasmic blebs or pseudopod formation. Cytochemically, they are negative for myeloperoxidase and positive for platelet peroxidase. Megakaryoblasts express CD41 (glycoprotein IIb/IIIa) and/or CD61 (glycoprotein IIIa)

TABLE 12.12. FAB classification of acute lymphoid leukaemias (ALL)

L1	85%	**Morphology:** L1 blasts are small and homogeneous. The nuclei are round and regular with little clefting and inconspicuous nucleoli. Cytoplasm is lightly basophilic, scanty and usually without vacuoles
		Staining: MPO is always negative
		Maturation: pro-B or pre-B lineage
L2	14%	**Morphology:** L2 blasts are large and heterogeneous. The nuclei are irregular and often clefted. One or more, usually large nucleoli are present. The volume of cytoplasm is variable, but often abundant and may contain vacuoles
		Cytochemistry: L2 blasts may have granular PAS positivity. MPO is negative
		Maturation: pro-B or pre-B and T-cell ALL lineage
L3	Burkitt's 1%	**Morphology:** L3 blasts are large in size and homogeneous. The nuclei are regular and round-oval in shape. One or more prominent nucleoli are present. They have moderate to abundant deeply basophilic cytoplasm, which contains prominent vacuoles
		Cytochemistry: MPO is always negative. NSE is usually negative, but may show focal cytoplasmic positivity. Vacuoles are PAS-negative but are classically positive for the neutral lipid stain Oil Red O
		Maturation: All L3 leukaemias are surface immunoglobulin (SIg)-positive and are of B-cell lineage

(b) Chronic leukaemias

Chronic leukemias

Chronic lymphocytic
- Common B cell
- Rare T cell
- Hairy cell
- Prolymphocytic

Chronic myelocytic (myeloid)
- Ph* positive
- Ph* negative, BCR** positive
- Ph* negative, BCR** negative
- Eosinophilic leukaemia

*Philadelphia chromosome.
**Breakpoint cluster.

2. **World Health Organization (WHO) classification of acute leukaemias**
   Blast count for diagnosis of acute leukaemia > or = 20% in peripheral blood or bone marrow (in FAB classification the cut off is 30%; it has been demonstrated that the survival pattern of patients with 20–30% blasts is similar to those with a count of >30%).

WHO classification of AML

- AML with recurrent genetic abnormalities
  - AML with t(8; 21) (q22; q22); AML1/ETO
  - AML with abnormal bone marrow eosinophils inv(16)(p13; q22) or t(16; 16)(p13; q22); (CBFβ/MYH11)
  - Acute promyelocytic leukaemia AML with t(15; 17)(q22; q12)(PML/RARα) and variants
  - AML with 11q23(MLL) abnormalities
- AML with multilineage dysplasia
  - Following a myelodysplastic syndrome
  - Without antecedent myelodysplastic syndrome
- AML and myelodysplastic syndromes, therapy related
  - Alkylating agent related
  - Topoisomerase Type II inhibitor related
  - Other types
- AML not otherwise characterized/specified
  - AML minimally differentiated
  - AML without maturation
  - AML with maturation
  - Acute myelomonocytic leukaemia
  - Acute monoblastic and monocytic leukaemia
  - Acute erythroid leukaemia
  - Acute megakaryoblastic leukaemia
  - Acute basophilic leukaemia
  - Acute pan myelosis with myelofibrosis
  - Myeloid sarcoma
  - Myeloid proliferations related to Down's syndrome
  - Blastic plasmacytoid dendritic cell neoplasms

Classification of ALL (Table 12.13)

TABLE 12.13. Classification of ALL

WHO type	FAB correlation
Precursor B lymphoblastic leukaemia/lymphoma	L1 and L2
Precursor T lymphoblastic leukaemia/lymphoma	L1 and L2
Leukemic phase of Burkitt lymphoma	L3

Q. Write briefly on the aetiopathogenesis of leukaemias.

Ans. The factors contributing to the etiopathogenesis of leukemias are:

- Familial and genetic: Down syndrome, ataxia telangiectasia, Fanconi anaemia and Bloom syndrome
- Drugs and toxins: Cytotoxic drugs like alkylating agents and exposure to benzene
- Retroviruses: Human T-cell leukaemia-lymphoma virus (human T-cell lymphotropic Type I virus)
- Ionizing radiation: Therapeutic irradiation, diagnostic X-rays and nuclear bombs
- Immunological: Immunodeficiency states

Q. Define acute leukaemia. Outline its clinical features and laboratory diagnosis.

Ans. Acute leukaemia is characterized by the replacement of normal marrow elements by immature cells called leukaemic blasts, which ultimately spill over into the peripheral blood.

Clinical Features

The clinical presentation of acute leukaemias is due to one or more of the following:

Anaemia

- Pallor, tiredness, malaise and effort intolerance
- Cardiorespiratory symptoms in severe anaemia

Granulocytopenia

Infections at various sites, eg, upper respiratory tract, skin, gingiva, lungs and urinary tract. Superficial lymphadenopathy and fever are common.

Thrombocytopenia

- Causes bleeding from gum, nose (epistaxis), skin (purpura, ecchymoses, petechiae and easy bruising), GIT, renal tract and uterus. Bleeding into eye and ear is also seen.
- Intracranial bleeding is a serious and fatal complication.

Myeloid Proliferation

Causes expansion of marrow leading to bone pains and sternal tenderness.

Leukaemic Infiltration Into Organs

- Common in liver, spleen and lymph nodes; results in hepatosplenomegaly and generalized lymphadenopathy
- Involvement of the central nervous system results in infiltration of brain parenchyma and meninges ('leukaemic meningitis')
- Other areas of leukaemic infiltration include mouth, gums (causing gingival hypertrophy), skin, testes, ovaries, eyes and bone.
- Localized proliferation of myeloblasts outside marrow produces solid tumours called **chloromas**.

Diagnosis

- **Morphological examination of blood and bone marrow shows**
  - Severe anaemia of the normocytic normochromic type
  - A markedly raised TLC (range 1×10^9/L to 500×10^9/L)
  - Numerous blast cells in the peripheral smear
  - Markedly decreased platelet count
  - Hypercellular bone marrow with replacement of normal elements by leukemic blast cells
- **Cytochemical stains in acute leukaemias**
  - Myeloperoxidase (MPO) is used for identification of primary or azurophilic granules in myeloid precursors. Positive in AML-M1, -M2, -M3 and -M4.
  - Staining with Sudan black B (SBB) and chloroacetate esterase (CAE) is mostly similar to myeloperoxidase. These stain the phospholipids in the membrane of neutrophilic granules.
  - Nonspecific esterase (NSE) is present in large quantities in monocytic cells and is positive in AML-M4 and -M5.

- Periodic acid-Schiff (PAS) shows granular positivity in the erythroblasts of M6 as well as block positivity in blasts of L1 and L2 subtypes of ALL.
- Acid phosphatase shows a strong focal positivity in T-cell ALL.

- **Immunophenotyping in acute leukaemias**
  Primary panel (to distinguish AML from ALL and classify B-ALL and T-ALL)
  - Myeloid: CD13, CD33 and CD117
  - B Lymphoid: CD19, CD79a(cyt), CD22(cyt) and CD10
  - T Lymphoid: CD3(cyt), CD2 and C7
  - Nonlineage restricted (primitive stem cell): HLA-R, TDT and CD34

  Secondary panel (to diagnose AML of monocytic, erythroid, megakaryocytic lineage and further subtyping of B and T-cell ALL)
  - Myeloid: CD14, CD64, lysozyme, glycophorin A, CD41 and CD61
  - B Lymphoid: cytIgM, surface Ig (κ/λ)
  - T Lymphoid: CD1a, membrane CD3, CD5, CD4 and CD8
- **Common cytogenetic abnormalities in acute leukaemias are listed in** Table 12.14.

TABLE 12.14. Common cytogenetic abnormalities in acute leukaemias

Chromosomal abnormality	Type of leukaemia	Prognosis
t(8;21)(q22;q12)	AMLM2	Favourable
t(15;17)(q22;q12)	AMLM3	Favourable
Inv(16)(p13;q32)	AMLM4E0	Favourable
Abnormalities of 11q23	AML monocytic	Intermediate
del(7q), del(5q), +8, +9, del(11q)	AML with multilineage dysplasia, therapy-related AML	Unfavourable
t(9;22)(q34;q11.2)	Precursor B ALL	Unfavourable
t(4;11)(q21;q23)	Precursor B ALL	Unfavourable
t(1;19)(q23;q13.3)	Precursor B ALL	Unfavourable
t(12;21)(q13;q22)	Precursor B ALL	Favourable
Hyperdiploidy	Precursor B ALL	Favourable
Hypodiploidy	Precursor B ALL	Unfavourable

Q. Differentiate between acute lymphoblastic and acute myelogenous leukaemia (or differentiate between lymphoblast and myeloblast).

Ans. Differences between acute lymphoblastic and acute myelogenous leukaemia are listed in Table 12.15.

TABLE 12.15. Differences between acute lymphoblastic and acute myelogenous leukaemia

Features	Acute lymphoblastic leukaemia (ALL)	Acute myelogenous leukaemia (AML)
Clinical features		
• Age group	Children	Adults
• Lymphadenopathy	Prominent	Less prominent
• Hepatosplenomegaly	50–75%	Less common
• CNS involvement	More common	Less common
• Gum involvement	Not seen	Gum hypertrophy common in M5 type
• Testicular involvement	In 10–20%	Not seen
• Eye involvement	More common	Less common
• Bleeding manifestations	Less common	More common

Continued

TABLE 12.15. Differences between acute lymphoblastic and acute myelogenous leukaemia—cont'd

Features	Acute lymphoblastic leukaemia (ALL)	Acute myelogenous leukaemia (AML)
Investigations		
• Leukemic blasts	Lymphoblasts (Fig. 12.5)	Myeloblasts (Fig. 12.6)
• Size	Smaller, 10–15 microns	Larger, 12–20 microns
• N/C ratio	High	Low
• Chromatin	Clumped	Spongy, skein like
• Nucleoli	< 2; indistinct	2–5; distinct
• Nuclear membrane	Irregular, convoluted	Regular
• Auer rods	Not present	Present in 10–20%
• TdT (terminal deoxynucleotidyl transferase)	Often positive	Negative
Cytochemical staining		
• Myeloperoxidase	Negative	Positive
• Sudan black B	Negative	Positive
• Chloroacetate esterase	Negative	Positive
• Periodic acid–Schiff (PAS)	Positive (shows block pattern)	Positive in < 25% of cells

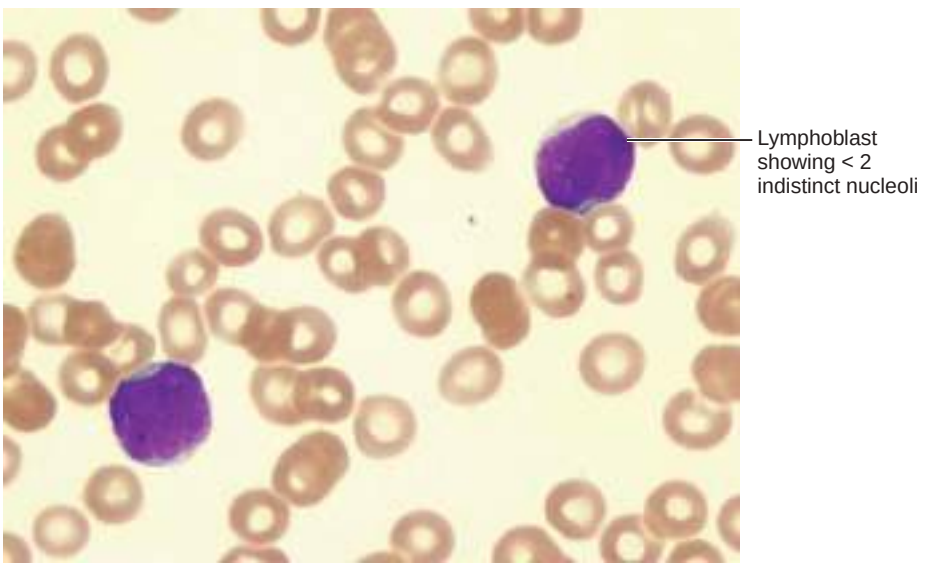


FIGURE 12.5. PBS from ALL L2 showing lymphoblasts (smaller cells, 10–15 microns in size with a high N/C ratio; clumped nuclear chromatin; <2 indistinct nucleoli and irregular to convoluted nuclear membrane).

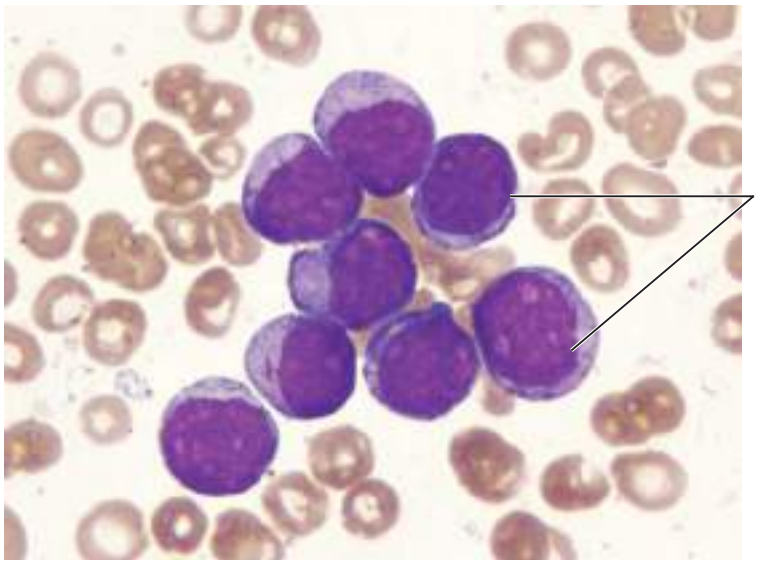


FIGURE 12.6. PBS from AML showing numerous myeloblasts (larger cells, 12–20 microns in size with a low N/C ratio, spongy, skein-like chromatin; 2–5 distinct nucleoli; regular nuclear margins).

Q. Define subleukaemic or aleukaemic leukaemia.

Ans. In some patients with acute leukaemia, the total leukocyte count is normal or less than normal but abnormal cells are seen in the peripheral blood; this is termed **subleukaemic leukaemia**. In about 10% of the patients with acute leukaemia, total leukocyte count is normal or less than normal and there are no abnormal cells in the peripheral blood. This is called **aleukaemic leukaemia.** Diagnosis is confirmed by examining the bone marrow, which shows a larger number of leukaemic cells.

Q. Discuss the clinical features and laboratory diagnosis of chronic myeloid leukaemia (CML).

Ans. CML is a myeloproliferative disease characterized by excessive proliferation of myeloid cells with near normal maturation.

Natural Course

The disease has three phases:

1. **Chronic stable phase**

Symptoms

- Peak incidence in 4th and 5th decades
- Patients may be asymptomatic in the early stage. Symptoms are mainly due to massive splenomegaly, anaemia and a hypermetabolic state.
- Symptoms due to massive splenomegaly include abdominal distension, dyspepsia, flatulence, reflux oesophagitis, dyspnoea and dragging discomfort in the left hypochondrium.
- Hepatomegaly may be seen.
- Symptoms resulting from the hypermetabolic state include fever, weight loss, night sweats and heat intolerance.
- Anaemia manifests as fatigue, weakness and anorexia.
- Priapism (high counts leading to obstruction of flow in the corpus cavernosum) may be seen.
- Bleeding tendencies occur late.

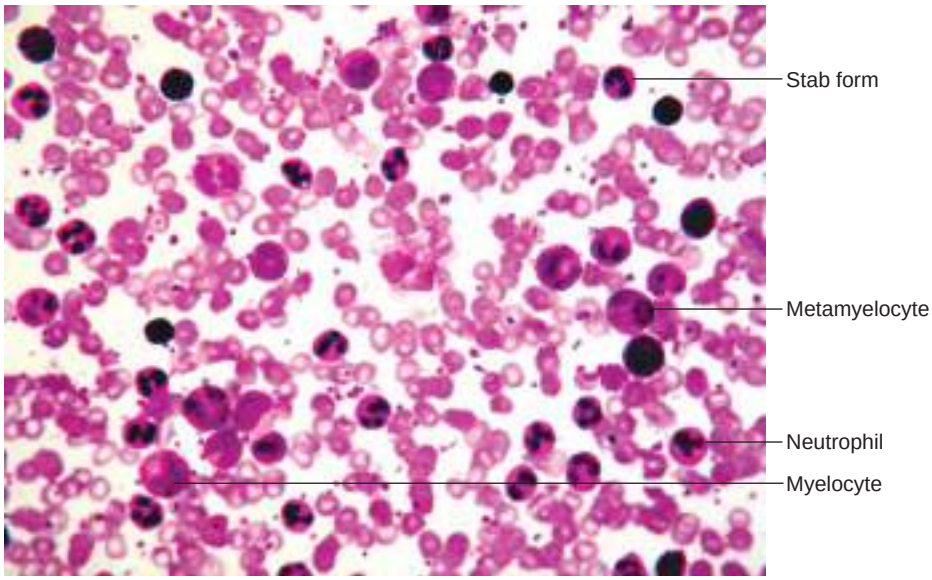


FIGURE 12.7. PBS showing a markedly raised leukocyte count with granulocyte precursors ranging from myeloblasts, myelocytes and metamyelocytes to mature neutrophils. Segmented neutrophils and myelocytes predominate.

Laboratory diagnosis:

- ***Peripheral smear*** (Fig. 12.7)
  - Normocytic normochromic anaemia
  - Total leukocyte count is markedly raised, typically between 100×10^9 and 300×10^9/L.
  - Granulocyte precursors ranging from myeloblasts, myelocytes and metamyelocytes to mature neutrophils are seen. Segmented neutrophils and myelocytes predominate.
  - Myeloblasts are less than 10%.
  - Increase in basophils and eosinophils is observed.
  - Platelets are normal or increased.
- ***Bone marrow***
  - Hypercellular bone marrow with marked proliferation of all granulocytic elements
  - Twenty to thirty percent of patients show mild bone marrow fibrosis in late stages.
- ***Philadelphia chromosome (Ph)*** is positive in more than 95% of cases, in all three phases. This is a reciprocal translocation between the long arms of chromosome 9 and chromosome 22 (t9; 22).
- ***Leukocyte alkaline phosphatase (LAP) score*** is very low, usually less than 5 (normal 20–100).

2. **Accelerated phase of CML:** CML may transform itself to a blastic phase with or without going through an accelerated phase. Features of accelerated phase are the following:
   (a) Progressive anaemia
   (b) Increase in splenic size
   (c) Increase in total leukocyte count with an increase in circulating immature cells (blast cells 10–19% in the peripheral blood/or bone marrow)
   (d) Peripheral blood basophilia (> 20%)
   (e) Persistent thrombocytosis (> 1,000,000/mm^3) or thrombocytopenia (< 100,000/mm^3) not responsive to therapy
   (f) Cytogenetic evidence of clonal evolution (cytogenetic changes in addition to Ph chromosome, eg, trisomy 8, etc.)

3. **Blast crisis phase of CML:** This phase represents the transformation of CML into an acute leukaemia.
   (a) 'Myeloid blast' crises (70%) when the disease transforms into acute myeloblastic leukaemia.
   (b) 'Lymphoid blast' crises (30%) when the disease transforms into acute lymphoblastic leukaemia.

Blast crisis is characterized by

- Sudden increase in splenic size
- Anaemia and thrombocytopenia
- Generalized lymphadenopathy
- Peripheral smear and bone marrow showing numerous blast cells (> 20%) simulating acute leukaemia
- Refractoriness to treatment (treatment of blast crisis is as for acute myeloblastic or lymphoblastic leukaemia)

Q. Outline the clinical features and laboratory diagnosis of primary myelofibrosis (agnogenic myeloid metaplasia).

Ans. Myelofibrosis is a clonal myeloproliferative disorder characterized by increased fibrosis within the marrow, splenomegaly and extramedullary haemopoiesis in the spleen, liver and at times, in lymph nodes, kidneys and adrenals.

Clinical Features

It is commonly seen between 40 and 70 years manifests with:

- Symptoms of **anaemia** like lassitude, fatigue, weakness and anorexia
- Symptoms due to **massive splenomegaly** like abdominal distension, dyspnoea and dragging discomfort in the left hypochondrium
- Symptoms resulting from **hypermetabolic state** like fever, weight loss, sweating and heat intolerance
- In late stages, bleeding tendencies occur due to **thrombocytopenia**.
- Hepatomegaly with portal hypertension and oesophageal varices, lymphadenopathy, ascites, cardiac failure and jaundice also seen

Laboratory Diagnosis

- Typical picture is **leukoerythroblastic** (simultaneous presence of erythroid and granulocytic precursors in the peripheral blood). There is marked anisopoikilocytosis. RBCs are usually normocytic normochromic with the presence of a fair number of tear-drop poikilocytes and oval/elliptical cells. Polychromatophils and basophilic stippling may also be seen.
- Platelet count is increased in the early stages, but decreased in the late stages.
- Total leukocyte count may be normal, increased (early stages) or decreased (late stages). Myeloid precursors are abundant but blasts do not exceed 10%.
- ***Bone marrow examination***
  - **Early stage** or **'cellular phase':** The marrow is hypercellular with an increase in all three cell lines, particularly megakaryocytes. Fibrosis is minimal.
  - **Late stage** or **'hypocellular phase'**: The marrow is hypocellular with reduction in all cell lines. Marked increase in fibrosis.
  - **Leukocyte alkaline phosphatase (LAP) score** is elevated.
  - **Philadelphia chromosome** is negative.

Q. Differentiate between CML and myelofibrosis.

Ans. Contrasting features of CML and myelofibrosis are listed in Table 12.16.

TABLE 12.16. Comparison between CML and myelofibrosis

Features	CML	Myelofibrosis
Clinical features		
• Splenomegaly	Moderate to marked	Marked
• Fever	Common	Uncommon
Laboratory investigations		
• RBCs	• Marked anaemia • Mild poikilocytosis	Slight to moderate anaemia Prominent poikilocytosis with tear-drop cells
• WBCs	Marked increase; 20–50 × 10^9/L	Normal, raised or low; when raised not more than 50 × 10^9/L
• Nucleated red cells	Few if any	Numerous
• LAP	Low	Normal, raised or reduced
• Bone marrow aspiration	Hyperplastic marrow with absence of fat spaces	Dry tap without marrow fragments
• Chromosomal analysis	Philadelphia-positive	Philadelphia-negative

Q. Write briefly on myelodysplastic syndrome (MDS).

Ans. The myelodysplastic syndromes are clonal disorders characterized by ineffective haematopoiesis and production of defective haematopoietic cells of erythroid, myeloid and megakaryocytic series. These patients are at increased risk of developing acute leukaemias.

Aetiology

In most cases the cause is unknown (idiopathic MDS); however, exposure to radiation, cancer chemotherapy, pesticides and ageing are implicated.

Clinical Features

- Failure of bone marrow to produce normal blood cells leads to anaemia, leucopenia and thrombocytopenia.
- Extramedullary haematopoiesis may occur leading to hepatomegaly and splenomegaly.

Classification (see Table 12.17)

TABLE 12.17. Classification of MDS

Category	Criteria
Refractory anaemia (RA)	• Anaemia with reticulocytopenia • Normal or hypercellular bone marrow with dyserythropoiesis; blasT cells < 5%
Refractory anaemia with sideroblasts (RARS)	• Same as refractory anaemia with ringed sideroblasts (> 15% of nucleated marrow cells)
Refractory anaemia with excess blasts (RAEB)	• Cytopenia of two or more cell lines with morphologic abnormalities of blood cells • Hypercellular bone marrow with blasts 5–20% of nucleated marrow cells
Refractory anaemia with excess blasts in transformation (RAEBT)	• Cytopenia of two or more cell lines with morphologic abnormalities of blood cells and > 5% blasts in peripheral smear • Hypercellular bone marrow with blasts 20–30% of nucleated marrow cells • Auer rods in granulocyte precursors
Chronic myelomonocytic leukaemia	• Cytopenia of two or more cell lines with morphologic abnormalities of blood cells and absolute monocytosis • Significant increase in marrow monocyte precursors

Laboratory Diagnosis

- Anaemia with macrocytosis and anisocytosis; RBCs may be dimorphic in RARS (both hypochromic and normochromic cells seen)
- Thrombocytopenia (platelets vary in size and some appear hypogranular)
- The WBC count may be normal, increased or decreased. Hypogranular or agranular granulocytes and neutrophils with bilobed Pelger Huet anomaly may be seen.
- Marrow is normocellular to hypercellular. Erythroid precursors show dyserythropoiesis. Immature myeloid cells are present in less well-differentiated subgroups (refractory anaemia with excess of blast cells and refractory anaemia in transformation).

LYMPHORETICULAR SYSTEM

Nonneoplastic Proliferations of Lymph Nodes

Q. What is reactive lymphadenitis?

Ans. Infections and noninfectious inflammatory stimuli can cause lymphadenitis, which may be classified as:

Acute Nonspecific Lymphadenitis

- May be confined to a local group of lymph nodes draining a focal infection
- May be generalized in systemic bacterial or viral infections

Gross Morphology

- Tender and fluctuant in case of abscess formation
- Involvement of the overlying skin can produce draining sinuses

Microscopy

- Large germinal centres
- A neutrophilic infiltrate is seen about the follicles and within lymphoid sinuses in pyogenic infections
- In severe infections, centres of the follicles undergo necrosis resulting in formation of an abscess

Chronic Nonspecific Lymphadenitis

Assumes three patterns depending on the causative agent, namely, follicular hyperplasia, paracortical hyperplasia and sinus histiocytosis

1. **Follicular hyperplasia**
   (a) Associated with infections and inflammations, which activate B cells
   (b) Follicles are enlarged with prominent germinal centres.
   (c) Cells in the reactive follicles include activated B cells, scattered macrophages containing nuclear debris **(tingible body macrophages)** and follicular dendritic cells
   (d) May be confused with follicular lymphomas

Features favouring a diagnosis of follicular hyperplasia over a follicular lymphoma

- Preservation of lymph node architecture with normal areas between germinal centres
- Variation in shape and size of lymphoid nodules
- Mixed population of lymphocytes at various stages of differentiation
- Prominent phagocytic and mitotic activity in germinal centres

2. **Paracortical hyperplasia**
   (a) Reactive changes in the T-cell regions
   (b) Encountered in viral infections (EBV), following vaccinations (small pox) and in drug reactions (phenytoin)
3. **Sinus histiocytosis**
   (a) Distension of the lymphatic sinusoids
   (b) Hypertrophy of lining endothelial cells and increase in the number of macrophages
   (c) Often seen in lymph nodes draining cancers (immune response to tumour and its products)

Q. Enumerate the causes of generalized lymphadenopathy.

Ans. ***Causes of generalized lymphadenopathy***

- Disseminated tuberculosis
- HIV-associated lymphadenopathy
- Secondary syphilis
- Infectious mononucleosis
- Brucellosis
- Systemic lupus erythaematosus and rheumatoid arthritis
- Lymphomas
- Leukaemias (ALL and CLL)

Neoplastic Proliferations of Lymph Nodes

Q. Write in detail on Hodgkin lymphoma (HL).

Ans. HL has a bimodal age incidence; affects young adults (15–35 years) and older adults (45–75 years). **Reed–Sternberg (RS) cells** are the diagnostic hallmark.

Classification

- Nodular sclerosis (NS)
- Mixed cellularity (MC)
- Lymphocyte rich (LR)
- Lymphocyte depleted (LD)

- *NS, MC, LR & LD are also called "classical HL".*
- *All have RS cells with similar phenotype, positive for PAX5 (a B cell transcription factor), CD15 and CD30 and negative for other markers.*

- Lymphocyte predominant (LP) → B-cell immunophenotype of RS cells (positive for CD20 and BCL6 and negative for CD15 and CD30).

RS Cell (Fig. 12.8)

- Large cell (15–45 microns) with abundant cytoplasm
- Classically has a bilobed mirror image nucleus
- Multiple nuclei or single nucleus with multiple lobes may be seen
- Nucleus typically has a large inclusion-like nucleolus of the size of a small lymphocyte (5–7 microns)

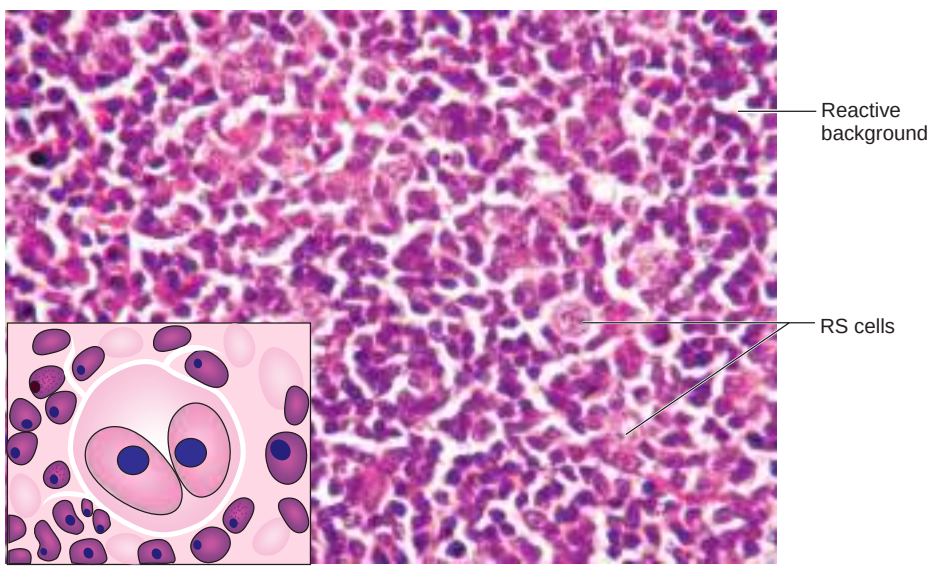


FIGURE 12.8. RS cell showing abundant cytoplasm and a bilobed mirror image nucleus and a large inclusion-like nucleolus of the size of a small lymphocyte (H and E; 400×).

Variants of RS Cells

1. Mononuclear variants: Single round to oblong nucleus with a large inclusion-like nucleolus
2. Lacunar cells: Predominantly seen in NS subtype. Delicate, folded and multilobated nucleus with abundant pale cytoplasm often disrupted while cutting sections. Nucleus appears to be sitting in a hole (lacuna).
3. L and H variants:
   RS cells undergo mummification (shrinkage and pyknosis) to give rise to cells with polypoid nuclei resembling popcorn, having inconspicuous nucleoli and moderate to abundant cytoplasm. Usually seen in the LP subtype.

Note: RS-like cells may be seen in solid cancers, non-Hodgkin lymphoma and infectious mononucleosis. **For diagnosing 'HL', RS cells must be present in a background of non-neoplastic cells (lymphocytes, plasma cells and eosinophils).**

Aetiology and Pathogenesis

- The *cell of origin of RS cells is thought to be a germinal centre or postgerminal centre B lymphocyte.*
- Rarely (1–2% cases) RS cells have TCR rearrangements suggesting *origin from transformed T cells.*
- EBV episomes are frequently present in RS cells. EBV-positive tumour cells express latent membrane protein or LMP-1 (a protein encoded by EBV genome that has transforming activity).
- LMP-1 *upregulates NF-KB* (transcription factor responsible for lymphocyte activation).
- NF-KB activation appears to be a common event in classical EBV-positive HL (NF-KB activation in EBV-negative cases occurs by acquired mutation in a negative regulator IKB).
- NF-KB activation possibly rescues cells from apoptosis.
- Accumulation of reactive cells is thought to be in response to cytokines released by RS cells, eg, IL-5, IL-6, IL-13, TNF and GM CSF.

Clinicopathological Features of Hodgkin Lymphoma (Table 12.18)

TABLE 12.18. Clinicopathological features of Hodgkin lymphoma

Subtypes	Morphology	Immunophenotype	Clinical features
NS	Frequent 'lacunar cells' and occasional diagnostic RS cell; background of T lymphocytes, eosinophils, macrophages and plasma cells. Fibrous bands divide cellular areas into nodules; cells arranged in syncytial sheets with interspersed necrosis	RS cells are CD15-and 30-positive; EBV-negative	• Mediastinal involvement is commonly seen • Most patients present in Stage I or II of the disease • F = M; affects young adults • Constitutes 65–75% of HL
MC	Frequent 'mononuclear' and 'diagnostic RS cells'; background infiltrate rich in T lymphocytes, eosinophils, macrophages, plasma cells	RS cells are CD15-and 30-positive; 70% EBV-positive	• > 50% present as Stage III or IV disease • Usually involve neck nodes • M > F/biphasic age distribution seen in young adults and > 55 years • Constitute 20–25% of HL
LR	Frequent 'mononuclear' and 'diagnostic RS cells', background rich in T lymphocytes	RS cells are CD15-and 30-positive; 70% EBV-positive	• Uncommon • M > F • Affects older adults

Continued

TABLE 12.18. Classification of Hodgkin lymphoma—cont'd

Subtypes	Morphology	Immunophenotype	Clinical features
LD	**Reticular variant:** Many 'diagnostic RS cells' and 'variants' with paucity of background reactive cells. **Diffuse fibrosis variant:** Hypocellular fibrillar background with scattered 'diagnostic RS cells' and 'variants' and few reactive cells	RS cells are CD15 and 30-positive; EBV-positive	• Affects older males • Frequent association with HIV infection • Usually present with advanced disease. • Constitute < 5% cases of HL
LP	Frequent 'L and H (popcorn) cells' in the background of follicular dendritic cells and reactive B cells May transform into a large B cell lymphoma.	RS cells are CD 20-positive, CD15-negative, CD30-negative, EBV–BCL–6-positive (BCL6 is a germinal centre specific transcription factor)	• Young males • Cervical and axillary lymphadenopathy • Rarely involve mediastinal lymph nodes

Clinical Features

- Painless enlargement of lymph nodes, which are discrete, nontender and rubbery
- **'Constitutional symptoms'** (fever, night sweats, unexplained weight loss of greater than 10% body weight) are observed more with disseminated disease (Stages III and IV) and mixed cellularity or lymphocyte depletion subtypes.
- Classical **'Pel–Ebstein' fever** (fever showing cyclical pattern; several days or weeks of fever alternating with afebrile periods) is rare. An uncommon paraneoplastic symptom involves occurrence of pain in affected lymph nodes on consumption of alcohol.
- Cutaneous anergy due to depressed cell-mediated immunity may be seen.

Clinical Staging (Ann Arbor); (Table 12.19)

TABLE 12.19. Clinical Staging (Ann Arbor) of Hodgkin lymphoma

Stage	Distribution of disease
(I)	Involvement of single lymph node region (I) or involvement of a single extralymphatic organ or site (IE)
(II)	Involvement of two or more lymph node regions on the same side of diaphragm, alone (II) or with involvement of limited contiguous extralymphatic organs or tissue (IIE)
(III)	Involvement of lymph node regions on both sides of diaphragm, which may include spleen (IIIS) and/or limited contiguous extralymphatic organ/site (IIIE and IIIES)
(IV)	Multiple or disseminated foci of involvement of one or more extralymphatic organs or tissues with or without lymphatic involvement

Note: All stages are further divided on the basis of presence or absence of systemic symptoms into (A) and (B).
(*Source: Robbins and Cotran Pathologic Basis of Disease,* South Asia Edition, Vol II, Kumar, Abbas, Aster, Table 13-9, Page 611, Copyright 2015.)

Prognosis and Treatment

- Tumour stage rather than type more important prognostic factor
- With current treatment protocols, cure rates of **Stages I and II A**—90%
- **Advanced stage** associated with a 60–70% disease-free survival for 5 years
- Complications of long-term chemotherapy and radiotherapy:
  - **Neoplastic complications:** Increased rates of **myelodysplastic syndrome, acute leukaemia, lung cancer, NHL, breast** and **gastric carcinoma, sarcomas** and **malignant melanoma**
  - **Nonneoplastic complications:** Pulmonary fibrosis and accelerated atherosclerosis

Clinical differences between HL and NHL are given in Table 12.20.

TABLE 12.20. Clinical differences between HL and NHL

HL	NHL
More often localized to a single axial group of nodes	More frequent involvement of multiple peripheral nodes
Orderly spread by contiguity	Noncontiguous spread
Mesenteric nodes and Waldeyer ring rarely involved	Commonly involved
Extranodal involvement uncommon	Extranodal involvement common

Q. Write briefly on the epidemiology of non-Hodgkin lymphoma (NHL).

Ans. Malignant neoplasm of immune system:
- Approximately, 60% of malignant lymphomas are NHL, while remaining 40% are HL.
- Most primary malignancies arise in the lymph nodes; few are extranodal in origin.
- Stomach is the most common primary extranodal site.
- Low-grade lymphomas often metastasize to the bone marrow and peripheral blood (labelled leukaemic phase of the lymphoma).
- Immunohistochemical stains, identification of translocation and detection of Ig gene rearrangement are useful in the workup of NHL.
- Approximately, 60% of the patients with NHL are men over 50 years.

Q. Write briefly on the aetiopathogenesis of NHL.

Ans. NHL is characterized by clonal proliferation of immune cells. 65% of NHL are B-lymphocyte origin, 35% are T lymphocyte and 2% NK cell in origin.

Aetiologic factors implicated in the pathogenesis of NHL are

- Infections
  - *Helicobacter pylori* (MALT lymphoma of stomach)
  - EBV (Burkitt lymphoma, post-transplant lymphoma)
  - Human T-cell leukaemia virus Type I (adult T-cell lymphoma/leukaemia)
  - HIV (Diffuse large B-cell lymphoma, Burkitt lymphoma)
  - Hepatitis C (Lymphoplasmacytic lymphoma)
- Immunodeficiency diseases: Various inherited (ataxia telangiectasia, Wiskott–Aldrich syndrome) and acquired immunodeficiency diseases, eg, AIDS, iatrogenic immunosuppression induced by chemo or radiotherapy are implicated.
- Autoimmunity: Sjögren syndrome, nontropical sprue and rheumatoid arthritis are associated with a higher incidence of NHL.
- Chemical and drug exposure: Long-term exposure to phenytoin, agriculture chemicals, radiotherapy and chemotherapy
- Cytogenetic abnormalities: Chromosomal translocations, eg, overexpression of BCL-2 protein

Q. Classify NHL.

Ans. Classification systems used for classification of NHL:

- Rappaport
- Lukes–Collins
- Working formulation for clinical usage
- REAL
- WHO

1. **Rappaport** (1966)**:** Based on two features:
   (a) Low-power microscopy of the overall pattern of lymphoma
   (b) High-power microscopy and cytology of neoplastic cells
   Classifies NHLs into:
   (i) Nodular NHL
   - Lymphocytic, well differentiated

- Lymphocytic, poorly differentiated
- Lymphocytic and histiocytic mixed
- Histiocytic

(ii) Diffuse NHL
- Lymphocytic, well differentiated
- Lymphocytic, poorly differentiated
- Mixed lymphocytic and histiocytic
- Lymphoblastic
- Diffuse undifferentiated, Burkitt's and non-Burkitt's.

Disadvantages of Rappaport classification:
- No T-cell and B-cell subpopulation identification
- Cell of origin not identified

2. **Luke–Collins/Kiel classification (1974)**
   (a) Immunologic markers divide all lymphomas into B cell, T cell and, rarely, NK cell derived.
   (b) Sixty-five percent of NHL are B lymphocyte derived.
   Classifies NHLs into:
   (i) B-cell NHL
   - Small lymphocytic
   - Plasmacytoid lymphocytic
   - Follicular centre cell
   - Immunoblastic

   (ii) T-cell NHL
   - Small lymphocytic
   - Convoluted lymphocytic
   - Cerebriform
   - Immunoblastic

   (iii) Histiocytic NHL
   (iv) Undefined NHL

***Disadvantage of Luke–Collins/Kiel classification*:** Does not correlate with varying prognosis of different clinical types of NHL

3. **Working formulation for clinical usage (1982)**: Based on normal history of disease and long-term survival studies. Classifies NHLs into:
   (a) Low-grade NHL: 5-year survival is 50–70%.
   (i) Small lymphocytic
   (ii) Follicular and predominantly small cleaved
   (iii) Follicular, mixed small cleaved and large cleaved
   (b) Intermediate-grade NHL: 5-year survival is 35–45%.
   (i) Follicular and predominantly large cell
   (ii) Diffuse and small cleaved cell
   (iii) Diffuse mixed small and large cell
   (iv) Diffuse and large cell
   (c) High-grade NHL: 5-year survival is 25–35%.
   (i) Large cell immunoblastic
   (ii) Lymphoblastic
   (iii) Burkitt's

Disadvantage of Working formulation classification: No attempt is made to determine whether the tumour cells are B cell or T cell or macrophage in origin.

4. **Updated REAL (Revised European-American classification)/WHO classification (2008):** In 1994 REAL classification was proposed, however in view of the fact that it showed poor reproducibility, it is not used anymore. Since 1995, members of the European and American haematopathology societies have been collaborating on a new World Health Organization (WHO) classification of haematological malignancies. WHO classification uses an updated version of the REAL classification for lymphomas and extends the principles of the REAL classification to the classification of myeloid and histiocytic neoplasms. The REAL and WHO classifications recognize three major categories of lymphoid malignancies that can be defined on the basis of a combination of morphology and special studies that identify cell lineage: B-cell neoplasms, T-cell/natural killer (NK)-cell neoplasms and Hodgkin disease/Hodgkin lymphoma (HD).

(a) B-cell neoplasms
  (i) Precursor B-cell neoplasm: Precursor B-lymphoblastic leukaemia/lymphoma (B-ALL)
  (ii) Mature (peripheral) B-cell neoplasms
    - B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma
    - Lymphoplasmacytic lymphoma
    - Splenic marginal zone lymphoma (± villous lymphocytes)
    - Hairy cell leukaemia
    - Plasma cell myeloma/plasmacytoma
    - Extranodal marginal zone B-cell lymphoma of MALT type
    - Mantle cell lymphoma
    - Follicular lymphoma
    - Nodal marginal zone B-cell lymphoma (± monocytoid B cells)
    - Diffuse large B-cell lymphoma (NOS)
    - Diffuse large B cell lymphoma associated with chronic inflammation
    - Primary effusion lymphoma
    - Burkitt's lymphoma

(b) T-cell and NK-cell neoplasms
  (i) Precursor T-cell neoplasm: Precursor T-lymphoblastic lymphoma/leukaemia
  (ii) Mature (peripheral) T-cell neoplasms
    - T-cell prolymphocytic leukaemia
    - T-cell granular lymphocytic leukaemia
    - Chronic lymphoproliferative disorder of NK cells
    - Adult T-cell lymphoma/leukaemia (HTLV1+)
    - Extranodal NK/T-cell lymphoma and nasal type
    - Enteropathy-type T-cell lymphoma
    - Primary cutaneous T cell lymphoproliferative disorder
    - Mycosis fungoides/Sézary syndrome
    - Anaplastic large cell lymphoma
    - Peripheral T-cell lymphoma; unspecified (NOS)
    - Angioimmunoblastic T-cell lymphoma

Q. Write in detail about the gross and microscopic pathology of NHL.

Ans. Diagnosis made reliably on **lymph node biopsy**; FNAC not adequate for typing of NHL.

Gross

1. Lymph nodes are enlarged and matted.
2. Common groups involved are cervical, supraclavicular and axillary.
3. Cut surface is grey-white and fish-flesh like

Histopathology

Precursor B-cell and T-cell leukaemia/lymphoma (acute lymphoblastic leukaemia/lymphoma):

- Group of neoplasms composed of immature precursor B (Pre-B) or T (Pre-T) cells, referred to as lymphoblasts.
- Eighty-five percent of ALLs are precursor B-cell tumours that are aggressive and manifest as childhood acute leukaemia with symptoms relating to pancytopenia secondary to marrow involvement.
- Less common precursor T-cell ALLs (15% of childhood leukaemias) are also aggressive and manifest in adolescence with a thymic mass with variable splenic, hepatic and bone marrow involvement.

Morphology

- Normal tissue architecture is completely effaced by lymphoblasts having scanty cytoplasm and nuclei slightly larger than a small lymphocyte.

- Nuclear chromatin is condensed with inconspicuous nucleoli.
- Nuclear membrane may be convoluted or cleaved.
- Some transform to aggressive diffuse large B-cell lymphoma or prolymphocytic lymphoma.
- Precursor B cells are Tdt (terminal deoxytransferase) and CD19-positive immature B cells (variable expression of other B-cell markers).
- Precursor T cells are Tdt-positive immature T cells (CD2-, 7-positive and variable expression of other T-cell markers).

Karyotype

- Ninety percent have nonrandom karyotypic abnormalities
- Hyperdiploidy (>50 chromosomes) most common; associated with a good prognosis
- Poor outcome in pre-B-cell tumours is associated with translocation involving the MLL gene on chromosome 11q23 or Philadelphia chromosome positivity.
- Fifty-five to sixty percent of pre-T-cell tumours have activating point mutations in NOTCH1 (transmembrane receptor whose activity is essential for normal T-cell development, ie, proliferation and survival of pre-T cells).

Mature Peripheral B-Cell Malignancies

1. **Small lymphocytic lymphoma (SLL)/chronic lymphocytic leukaemia (CLL)**
   (a) CLL is diagnosed when there is persistent peripheral blood lymphocytosis exceeding 4000 cells/mm^3.
   (b) SLL is essentially a nodal disease (CLL and SLL are morphologically and genotypically similar; differ only in terms of peripheral blood involvement, in the absence of which, a diagnosis of SLL is rendered).
   (c) CLL more common than SLL in the western world
   (d) Both CLL and SLL uncommon in Asia

Pathophysiology:

- Neoplastic B cells suppress normal B-cell function resulting in hypogammaglobulinaemia.
- Simultaneously, 15% patients develop auto antibodies against their own RBCs.
- Tumour cells displace the normal marrow elements leading to anaemia, neutropenia and thrombocytopenia.

Morphology:

- Diffuse effacement of the lymph node architecture by sheets of small round lymphocytes (tumour cell is a resting lymphocyte with a dark staining nucleus and scanty cytoplasm showing minimal cytological atypia and mitoses)
- Absolute lymphocytosis in the peripheral blood with involvement of bone marrow, liver and spleen seen in almost all cases
- Neoplastic lymphocytes are fragile and frequently disrupted during preparation of smears; thus, labelled **smudge cells**
- Neoplastic cells are mature B cells expressing pan B-cell markers CD19, CD20, CD23 and surface immunoglobulin heavy and light chains along with CD5
- Fifty percent patients have karyotypic abnormalities, eg, trisomy 12 and deletions of chromosome 11 and 12.

2. **Follicular lymphoma**
   (a) More common in the western world than in Asian population
   (b) Affects older age group
   (c) Presents as painless generalized lymphadenopathy with or without visceral involvement
   (d) Lymph nodes effaced with a nodular appearance
   (e) Tumour cells resemble normal follicular centre B cells (centrocyte like with cleaved nuclear contours or nuclear infoldings, coarse chromatin and scanty cytoplasm). A few centroblast-like cells that have vesicular chromatin, several nucleoli and moderate cytoplasm, also seen.

(f) Pan B markers, CD19, CD20, CD10 and BCL6 (transcription factor required for follicular centre formation) and BCL2-positive
(g) Majority of tumours have a characteristic translocation t(14;18).
(h) Natural course of disease is prolonged (median survival from 7 to 9 years); but not easily curable due to increased levels of BCL2, which blocks apoptosis to increase survival of tumour cells.
(i) In 40% patients, follicular lymphomas progress to a diffuse large B-cell lymphoma.

3. **Mantle cell lymphoma**
   (a) Four percent of all NHLs
   (b) Present with lymphadenopathy and involvement of bone marrow, liver, spleen and bowel (multifocal submucosal nodules resembling polyps called lymphomatoid polyposis), mainly in older males
   (c) Lymph nodes show diffuse or vaguely nodular pattern of effacement
   (d) Composed of B cells that resemble the cells in the mantle zone of normal lymphoid follicles
   (e) Tumour cells are slightly larger than normal lymphocytes with an irregular nucleus and inconspicuous nucleoli and express IgM, IgD, CD19, CD20 and CD5.
   (f) Translocation (11;14) is commonly seen and results in fusion of Cyclin D1 gene on chromosome 11 to the IgH locus on chromosome 14 inducing dysregulation of expression of Cyclin D1 and increased levels of the same.
   (g) Aggressive with a median survival of 3–5 years
4. **Diffuse large B-cell lymphoma**
   (a) Accounts for 50% of adult NHLs
   (b) Median age 60 years; slight male predominance
   (c) This category includes several forms of NHL, which share the following features:
      (i) B-cell phenotype
      (ii) Diffuse growth pattern
      (iii) Aggressive nature (disseminate widely)
   (d) Pan B-cell markers positive along with surface IgM and IgG with variable expression of CD10.
   (e) Tumour cells are largely composed of cells that resemble **centroblasts** (3–4 times the size of resting lymphocytes, round, irregular-cleaved nuclear contours, dispersed chromatin, distinct nucleoli and moderate pale cytoplasm) as well as a few cells that resemble **immunoblasts** (large round to multilobated vesicular nucleus, 1–2 centrally placed prominent nucleoli and pale to intensely staining abundant cytoplasm).
   (f) Some patients have t(14;18); these tumours may represent 'transformed' follicular lymphomas.
   (g) A few cases show rearrangements/mutation in BCL6 gene leading to inappropriate increase in BCL6 protein.
   (h) Fatal if untreated; complete remission can be achieved in 60–80% patients by combination chemotherapy.
   (i) Several clinicopathological subtypes:
      (i) Diffuse large B-cell lymphoma that arises in the setting of AIDS and iatrogenic immunosuppression
      (ii) Diffuse large B-cell lymphoma arising in the posttransplant setting
      (iii) Kaposi Sarcoma herpes virus (KSHV) or Human herpes virus type 8 (HHV-8) associated 'primary effusion lymphomas' in the pleura, pericardium or peritoneum
      (iv) Mediastinal large B-cell lymphomas (arise in young females and frequently spread to abdominal viscera and central nervous system)
5. **Burkitt lymphoma**
   (a) This is a high-grade, non-Hodgkin lymphoma having small noncleaved cells.
   (b) Endemic in some parts of Africa and sporadic in the United States.
   (c) EBV plays an important aetiological role.
   (d) Majority of the cases occur in children; usually in extranodal sites.
   (e) In African patients, involvement of mandible and maxillary bones manifests with deformity, loosening of teeth and proptosis with loss of vision.

(f) The North American type preferentially involves the abdomen (bowel, retroperitoneum and ovaries).
(g) Tumour cells are uniform with round to oval nuclei (approximately, the size of nuclei of macrophages), 2–5 nucleoli and moderate amount of basophilic or amphophilic cytoplasm.
(h) Neoplasm characterized by a high mitotic rate and cell death leading to the presence of numerous macrophages with debris. These macrophages are often surrounded by a clear space creating a characteristic **'starry sky' appearance.**
(i) Tumour cells express surface IgM, kappa and lambda light chains and pan B-cell markers (CD19, CD20 and CD10).
(j) Chromosome analysis may show 8; 14 or 2; 8 or 8; 22 translocations. Most translocations fuse MYC with IgH gene on chromosome 14 resulting in dysregulation and overexpression of MYC protein.
(k) Antibodies to EB viral capsid antigen may be present.

6. **Mucosa-associated lymphoid tissue (MALT) lymphoma (extranodal marginal zone lymphoma)**
(a) Low-grade mature B-cell tumour that arises from mucosa-associated lymphoid tissue (MALT).
(b) Seen in salivary glands, stomach, small and large bowel, lungs, orbit and breast.
(c) Gastric type of MALT lymphoma is associated with *Helicobacter pylori* infection. Salivary gland MALT is associated with Sjögren syndrome indicating sustained antigenic stimulation may contribute to development of these lymphomas.
(d) It is mainly seen in elderly patients with median age of 60 years.
(e) MALT lymphomas may remain localized to the organ from which they arise or may spread to the surrounding lymph nodes.
(f) Bone marrow involvement is uncommon and occurs in only 15% cases. Distant metastasis is possible.
(g) Prognosis is good in most cases (5-year survival of 75%).

Common Mature Peripheral T-Cell Malignancies

1. **Mycosis fungoides and Sézary syndrome**
(a) Cutaneous T-cell lymphomas composed of neoplastic CD4+ T cells that home to the skin.
(b) The patient presents with chronic erythrodermic rash manifesting as a localized plaque-like lesion (plaque phase) which later becomes nodular and ulcerated (tumour phase).
(c) Histological findings include infiltration of the epidermis and upper dermis by neoplastic T cells, which have a cerebriform nucleus characterized by marked infolding of the nuclear membrane.
(d) In some cases, a leukaemic phase called **Sézary syndrome** appears, which is characterized by:
(i) Generalized exfoliative erythroderma
(ii) Tumour cells in the peripheral blood
(e) Patients with erythrodermic phase of mycosis fungoides usually survive for many years; survival less (1–3 years) for patients in tumour phase of the disease, visceral disease and Sézary syndrome.

2. **Adult T-cell lymphoma/leukaemia**
(a) Caused by a retrovirus, human T-cell lymphotropic virus-I (HTLV-I)
(b) Patients are infected through transplacental transmission, blood transfusion or sexual contact.
(c) Most patients have an aggressive disease characterized by lymphadenopathy, hepatosplenomegaly, skin infiltration, hypocalcaemia and lytic bone lesions.
(d) Peripheral smear usually reveals characteristic, pleomorphic abnormal CD4-positive cells with indented nuclei.
(e) Leukaemic cells express high levels of CD25 (The IL2 receptor α chain).
(f) Extremely aggressive disease with a median survival of 8 months. A few patients have long, chronic course.
(g) Combination chemotherapy may prolong life but does not produce remissions.

3. **Peripheral T-cell lymphoma (not otherwise specified or NOS)**
   (a) Refers to a group of diseases that do not fit into any of the other subtypes of PTCL (CD4 and CD8+, CD4>CD8; antigen loss frequent − CD7, CD5, CD4/CD8, CD52; CD30−/+, CD56−/+, CD10−, BCL6−, CLCX13−, PD1−).
   (b) It constitutes 25% of all PTCLs.
   (c) Its differential diagnosis includes:
      (i) Angioimmunoblastic lymphoma: CD4+ or mixed CD4/8, CD10+/−, BCL6+/−, CXCL13+, PD1+, hyperplasia of FDC, EBV+CD20+ B blasts
      (ii) Adult T cell leukaemia/lymphoma: CD4+, CD25+, CD7−, CD30−/+, CD15−/+, FoxP3+/−
      (iii) Anaplastic large cell lymphoma: CD30+, ALK+/−, EMA+, CD25+, cytotoxic granules+, CD4+/−, CD3−/+, CD43+
      (iv) T cell rich large B cell lymphoma: Large CD20+ blasts in background of reactive CD3+ T cells
      (v) T-zone hyperplasia: Mixed CD4/CD8, intact architecture, variable CD25 and CD30; scattered CD20+ B cells

Laboratory Findings in NHL

Haematological abnormalities:

1. Anaemia of normocytic normochromic type
2. Advanced disease with marrow infiltration →neutropenia, thrombocytopenia and leucoerythroblastic picture
3. Leukaemic conversion of NHL
4. Hyperuricaemia and hypercalcaemia late in the disease

Q. Outline the types, clinical features and laboratory diagnosis of chronic lymphocytic leukaemia (CLL).

Ans. CLL is characterized by a persistent lymphocytosis of at least 10×10^9 with infiltration of the bone marrow, spleen and lymph nodes.

Types

More than 95% of the cases are B-CLL; 5% are T-CLL

Clinical Features

- Most common form of chronic leukaemia in the Western world, usually seen in patients over 50 years; M > F.
- Patients may be asymptomatic or present with generalized lymphadenopathy and hepatosplenomegaly.
- Recurrent infections due to hypogammaglobulinaemia/synthesis of abnormal immunoglobulins and neutropenia.
- Haemorrhagic manifestations, eg, purpura, due to thrombocytopenia (impaired platelet production as normal marrow replaced by leukaemic cells, as well as immune destruction of platelets and hypersplenism).
- Constitutional symptoms due to raised metabolic rate (malaise, anorexia, weight loss, fever and night sweats)

Investigations

- Mild to moderate anaemia due to:
  (a) Marrow replacement by leukaemic cells
  (b) Autoimmune haemolysis
  (c) Folate deficiency
  (d) Hypersplenism
- Total leukocyte count is raised.

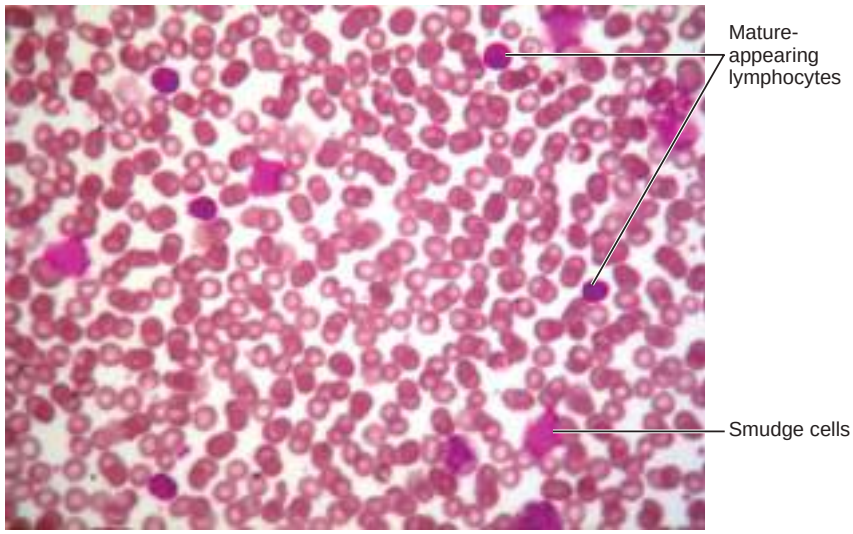


FIGURE 12.9. PBS of CLL showing mature-appearing lymphocytes with scanty, fragile cytoplasm, at places forming 'smudge' cells.

- More than 95% of the cells are small mature-appearing lymphocytes with scanty, fragile cytoplasm. Some of these are disrupted during preparation of the film and are called **'smudge, basket or smear' cells** (Fig. 12.9).
- Platelets are normal or reduced in number (autoimmune thrombocytopenia).
- Bone marrow is hypercellular with infiltration by tumour cells.
- Direct Coombs test may be positive indicating an autoimmune haemolytic process.
- Lymph node biopsy shows well-differentiated, small, noncleaved lymphocytes.
- Serum folate levels are low.

Clinical Staging (Binet Classification)

- Stage A
  - No anaemia or thrombocytopenia
  - Less than three areas of lymphoid enlargement
- Stage B
  - No anaemia or thrombocytopenia
  - Three or more areas of lymphoid enlargement
- Stage C
  - Anaemia and/or thrombocytopenia present, regardless of the number of areas of lymphoid enlargement

Lymphoid enlargement includes cervical, axillary, inguinal lymph nodes, liver and spleen.

Q. Outline the clinical features and laboratory diagnosis of hairy cell leukaemia.

Ans. ***Clinical features***

- Common in patients over 40 years, and more common in males.
- Symptoms are due to pancytopenia (mainly neutropenia and monocytopenia), massive splenomegaly and bleeding manifestations.

Investigations

- Normocytic normochromic anaemia with leucopenia and thrombocytopenia.
- Peripheral smear shows the characteristic hairy cells (B cells), which have an eccentrically placed nucleus, foamy cytoplasm and hairy cytoplasmic projections. These hairy cells stain positively for tartrate-resistant acid phosphatase (TRAP).
- Dry tap; biopsy shows fibrosis and infiltration by hairy cells.

- Splenic histology reveals mononuclear cell infiltration of red pulp and engorgement of sinuses.

Q. Enumerate various plasma cell disorders.

Ans. Plasma cell disorders are monoclonal neoplasms developing from common progenitors in the B-lymphocyte lineage. Also called paraproteinaemias and plasma cell dyscrasias, these include the following diseases:

- Multiple myeloma
- Waldenstrom macroglobulinaemia
- Primary amyloidosis
- Heavy chain disease

Q. Write in detail on the pathology, clinical features and laboratory diagnosis of multiple myeloma.

Ans. Clonal proliferation of plasma cells induced by the cytokine IL6, which is secreted by fibroblasts and macrophages in the bone marrow stroma.

- Plasma cells produce excessive immunoglobulins with only one type of light chain (kappa or lambda). These excess light chains are low molecular weight and appear in the urine as **Bence Jones proteinuria**.
- The immunoglobulin produced is called a 'paraprotein' (M-protein). It appears on electrophoresis as a clear-cut band (M-band or M-component).
- The most common M component is IgG (60%), followed by IgA (20–25%); only rarely is it IgM, IgD or IgE. In the remaining cases, the plasma cells produce kappa or lambda light chains only.

Clinical Features

- Peak incidence is between 60 and 70 years and males are more affected than females.
- ***Solitary bone plasmacytomas*:** Single lytic bone lesion without marrow plasmacytosis; present as punched-out lesions involving the flat bones (vertebrae, skull, sternum, ribs and clavicle). Manifest with bone pain, pathological fractures and compressive myelopathy (due to vertebral collapse and compression).
- ***Extramedullary plasmacytoma*:** Lesions in soft tissue (mainly in upper respiratory tract) without marrow plasmacytosis
- Those with skeletal plasmacytomas develop full-blown multiple myeloma over a period of 5–10 years; whereas, extraosseous plasmacytomas spread less commonly and are often cured by local resection.
- Serum uric acid is elevated due to increased cell turnover.
- Osteoclasts are stimulated resulting in bone resorption and generalized osteoporosis.
- Mobilization of calcium from bone results in hypercalcaemia, hypercalciuria and nephrocalcinosis.
- ***Renal involvement*:** Bence Jones proteinuria, amyloidosis, hypercalcaemia and hyperuricaemia result in renal damage and renal failure.
- ***Immune system involvement*:** Increased susceptibility to infections, particularly of the respiratory system and urinary tract.
- ***Hyperviscosity syndrome*:** Results from increased viscosity of blood. May cause blurred vision with retinal venous congestion, papilloedema, headache, vertigo, nystagmus, postural hypotension, congestive cardiac failure and clotting problems (purpura, epistaxis and gastrointestinal bleeding).
- May present with **neurological manifestations** (amyloid peripheral neuropathy, carpal tunnel syndrome, compressive myelopathy and radiculopathy)

Pathology

- Bone marrow is infiltrated heavily with atypical plasma cells. Gradual replacement of the marrow by plasma cells results in anaemia, leucopenia and thrombocytopenia (Fig. 12.10).

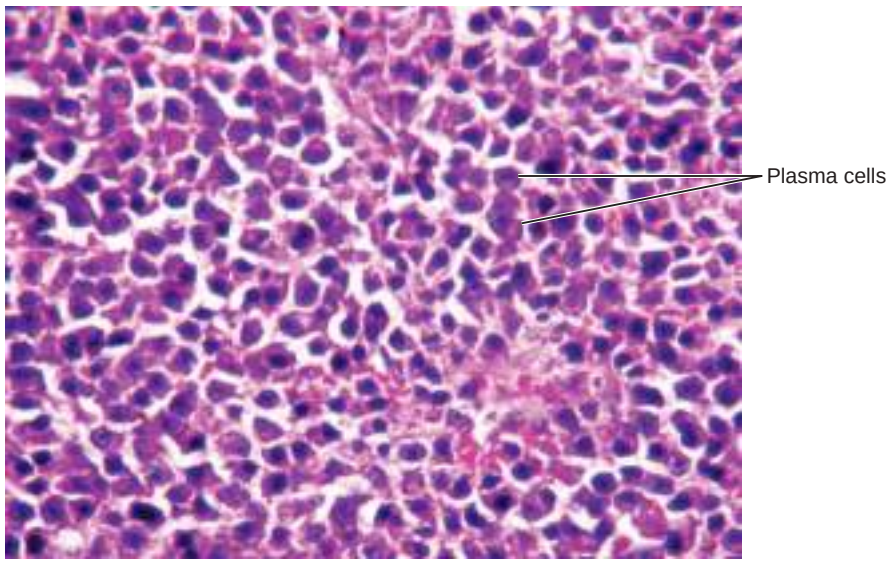


FIGURE 12.10. Bone biopsy of multiple myeloma showing sheets of plasma cells and precursors.

- In majority of patients, plasma cells are seen in the peripheral blood in small numbers. In a few patients, plasma cells are seen in the peripheral blood in significant numbers (more than 2000/mm^3), and this condition is known as '**plasma cell leukaemia**'.

Diagnosis

Major criteria

1. Plasmacytoma on tissue biopsy
2. Bone marrow shows greater than 30% plasma cells
3. Monoclonal globulin spike on serum protein electrophoresis with an IgG peak of $>$ 3.5/dL, IgA peak of $>$ 2 g/dL or urine protein electrophoresis result of $>$ 1 g/24 h

Minor criteria

(a) Bone marrow with 10–30% plasma cells
(b) Monoclonal globulin spike is present but less than major criteria 3
(c) Lytic bone lesions
(d) Residual IgM level is less than 50 mg/dL, IgA level less than 100 mg/dL or IgG level less than 600 mg/dL.

The following combination of findings are used to diagnose multiple myeloma:
1 plus b, c or d
2 plus b, c or d
3 plus a, c or d
a plus b plus C
a plus b plus d

Other important findings in multiple myeloma:

- Haemogram usually shows anaemia, leucopenia and thrombocytopenia with a raised ESR. Peripheral blood smear may show rouleaux formation.
- Bence Jones proteins may be present in the urine.
- Urea, creatinine and electrolytes should be done to assess renal function.
- Serum calcium and uric acid level are usually raised.
- Serum alkaline phosphatase is normal in the absence of complications.
- Total serum protein level is increased, albumin is decreased and globulins markedly increased.
- Serum β_2-microglobulin level may provide a useful assessment of prognosis. Higher levels indicate poor prognosis.

Q. Enumerate the common causes of splenomegaly.

Ans. ***Causes of splenomegaly***

- **Mild splenomegaly** (weight less than 500 g): Acute splenitis, acute splenic congestion, enteric fever, infectious mononucleosis, brucellosis, septicaemia, SLE, infective endocarditis, syphilis and parasitic infestations, eg, malaria, kala-azar.
- **Moderate splenomegaly** (weight 500–1000 g): Lymphomas, portal hypertension, acute leukaemias, chronic lymphocytic leukaemia, chronic myeloid leukaemia, haemolytic anaemias (hereditary spherocytosis and thalassaemia major), amyloidosis, Niemann–Pick disease, tuberculosis, sarcoidosis, typhoid, metastatic carcinoma and sarcoma.
- **Massive splenomegaly** (weight more than 1000 g): Chronic myeloid leukaemia, myelofibrosis, hairy cell leukaemia, tropical splenomegaly, kala-azar, portal hypertension, Gaucher disease, lymphomas, cysts and tumours of spleen.

Q. Write briefly on tropical splenomegaly.

Ans. Seen in residents of malaria endemic areas.

- Presents with low-grade fever and massive hepatosplenomegaly
- High levels of antimalarial antibodies are found in the blood. IgM levels are markedly raised.

Q. Write briefly on hypersplenism.

Ans. This is a term used to indicate anaemia, leucopenia and thrombocytopenia associated with prominent splenomegaly and a normal or hypercellular bone marrow (enlarged spleen removes excessive numbers of the formed elements of blood). Leucopenia and thrombocytopenia result from excessive sequestration of these cells in the large spleen. Anaemia is believed to be dilutional, resulting from an increase in total plasma volume.

Common Causes

- Primary hypersplenism: No detectable cause
- Secondary hypersplenism: Portal hypertension, malaria, kala-azar; topical splenomegaly syndrome and myeloproliferative disorders

Q. Write briefly on the mechanism of haemostasis.

Ans. There are three major components of the normal haemostatic mechanism:

1. **Vascular component:** This involves a reflex spasm of the injured vessel (vasoconstriction), which serves to minimize the blood loss.
2. **Platelet component**
   (a) Platelets are derived from marrow megakaryocytes. They are anucleate and have a discoid shape. The normal lifespan is about 10 days. About 70% of the platelets are in circulation while 30% are in the spleen.
   (b) The cytoplasm of platelets contains three major types of storage granules:
      (i) Alpha granules containing a variety of proteins like fibrinogen and von Willebrand factor
      (ii) Dense granules containing serotonin, ADP and calcium
      (iii) Lysosomal granules containing acid hydrolases

Following vessel constriction, platelets adhere to the vessel wall. This is facilitated by:

- Factor VIII/von Willebrand factor released from damaged endothelial cells
- Exposed subendothelial collagen
- Release of ADP and thromboxane A_2
- Platelet activation results in the discharge of the granule contents and formation of thromboxane A_2 from arachidonic acid, by the action of cyclooxygenase and thromboxane synthetase. Thromboxane A_2 is a potent stimulant of platelet aggregation.
- Platelet adhesion and platelet aggregation serve to form a platelet plug which seals off the vascular breach and arrests haemorrhage.

3. **Components of the coagulation cascade:** Coagulation cascade includes three 'pathways', namely, **intrinsic pathway, extrinsic pathway and common pathway.**

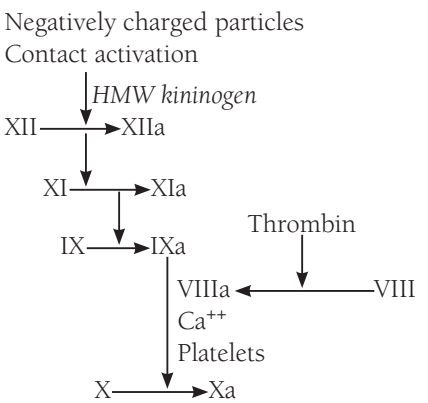
Intrinsic pathway is assessed in vitro by the activated partial thromboplastin time (aPTT). Extrinsic pathway is assessed by the prothrombin time (PT).

Coagulation Factors

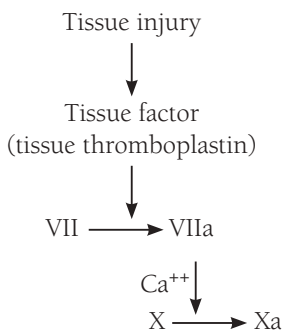
Factors	Traditional name
I	Fibrinogen
II	Prothrombin
V	Proaccelerin
VII	Proconvertin
VIII	Antihaemophilic factor
IX	Christmas factor
X	Stuart–Prower factor
XI	Plasma Thromboplastin antecedent
XII	Hageman factor
XIII	Fibrin stabilizing factor

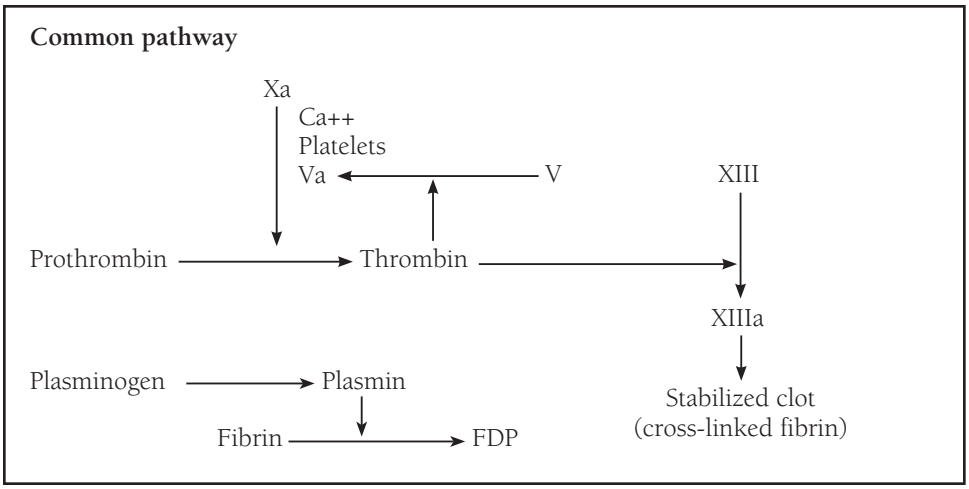
Prekallikrein: Fletcher factor.
HMW kininogen: Fitzgerald factor.

Intrinsic pathway



Extrinsic pathway





Inhibitors of Coagulation

The natural inhibitors of coagulation include

- Antithrombin III
- α-Macroglobulin
- Heparin cofactor II
- Protein C and protein S

Fibrinolytic System

The physiological function of the fibrinolytic system is to digest deposits of fibrin (thrombi).

Q. Write briefly on clinicopathological features of idiopathic thrombocytopenic purpura (ITP).

Ans. ITP, also known as primary immune thrombocytopenic purpura and autoimmune thrombocytopenic purpura, is defined as thrombocytopenia in the absence of other causes of thrombocytopenia.

Pathogenesis

- In ITP, an abnormal autoantibody, usually IgG with specificity for one or more platelet membrane glycoproteins (GPs), binds to circulating platelet membranes. In persons with chronic ITP, approximately 75% of autoantibodies are directed against platelet GPIIb/IIIa or GPIb/IX GP complexes.
- Autoantibody-coated platelets induce Fc receptor-mediated phagocytosis by macrophages in the spleen. Spleen is an important organ because platelet autoantibodies are also formed in the white pulp.

Clinical Features

- It manifests as a bleeding tendency, easy bruising (purpura); extravasation of blood from capillaries into skin and mucous membranes (petechiae and ecchymoses) or epistaxis; bleeding from the gums, haematuria, menorrhagia, metrorrhagia and melena are also seen.
- Cerebral haemorrhage is the most common cause of death in severe thrombocytopenia.
- ITP can be of two types, an acute self-limiting type and a chronic recurrent type.
- Adults may be affected at any age, but most cases are diagnosed in women aged 30–40 years. Peak age in children is 2–6 years.

Laboratory Diagnosis

- Haemogram shows thrombocytopenia with a normocytic normochromic anaemia (consequent to bleeding).
- Peripheral blood smear shows large/giant platelets, reflecting the early release of megakaryocytic fragments into the circulation. Platelets lack granules or have an abnormal colour. Lymphocytosis and eosinophilia are common.
- Tests for antiplatelet antibodies and assays for platelet-associated immunoglobulin, or antiplatelet antibodies are available.
- Bone marrow shows an increase in the number of megakaryocytes and their precursors which may show an abnormal morphology. There may be decreased cytoplasmic granularity, variable staining, vacuolization of the cytoplasm and hypolobulation of the nuclei.

Q. Differentiate between acute and chronic ITP.

Ans. Differences between acute and chronic ITP are listed in Table 12.21.

TABLE 12.21. Differences between acute and chronic ITP

Features	Acute ITP	Chronic ITP
Peak age affected	Children, 2–6 years	Adults, 20–40 years
Sex predilection	None	Female to male ratio 3:1
Prior infection	Common (1–3 weeks prior to onset)	Uncommon
Onset of bleeding	Abrupt	Insidious
Haemorrhagic bullae in the mouth	Present	Absent
Platelet count	$<20 \times 10^9$/L	$30–80 \times 10^9$/L
Eosinophilia and lymphocytosis	Common	Rare
Duration	2–6 weeks	Months to years
Spontaneous remission	Occurs in majority	Uncommon

Q. Classify hereditary coagulation disorders.

Ans. Classification of hereditary coagulation disorders:

1. **X-linked recessive traits**
   (a) Haemophilia A
   (b) Haemophilia B
2. **Autosomal recessive traits**
   (a) Factor XI deficiency
   (b) Prothrombin deficiency
   (c) Factors V/VII/X/XII/XIII deficiency
   (d) Afibrinogenaemia/Hypofibrinogenaemia
3. **Autosomal dominant traits**
   (a) von Willebrand disease
   (b) Dysfibrinogenaemias
   (c) Passovoy factor deficiency
4. **Combined**
   (a) Associated with haemophilia
   (b) Involving vitamin K-dependent factors
5. **Miscellaneous**
   (a) Prekallikrein deficiency
   (b) HMW Kininogen deficiency

Q. Write briefly on the pathophysiology of haemophilia.

Ans. Haemophilia is a frequently fatal haemorrhagic diathesis affecting male children characterized by a deficiency of Factor VIII (AHG and AHF).

Incidence

Incidence of hemophilia is 1 in 20,000 persons.

Pathophysiology

- Factor VIII normally circulates in the plasma bound to a much larger molecule VWF (VIII C along with VWF is called **Factor VIII complex**).
- Functional attribute of Factor VIII is VIII C.
- Examination of haemophilia genes has revealed seven different mutations.

Carrier Detection

- Demonstration of subnormal levels of Factor VIII C by immunoassays
- Ratio of VIII C to VWF normally 0.74–2.2; in carriers, 0.18–0.9
- Abnormally low ratios of VIIIC/VWF may be seen in stress
- Falsely high ratios of VIII C/VWF are seen in pregnancy, oral contraceptive intake and contamination of plasma samples with thrombin or proteolytic enzymes

Haemophilia in Females

- Minority of heterozygous carriers in whom X-chromosome inactivation has occurred
- Mating between affected male and carrier female
- Due to a new mutant gene in a carrier

Clinical Manifestations

- Excessive haemorrhage from a trivial injury
- Haemarthrosis most common and debilitating manifestation; usually preceded by 'Aura' (tingling before haemarthrosis)
- Haemorrhage → organization and inflammation → chronic proliferative synovitis → chronic haemophilic arthropathy (may lead to fibrous or bony ankylosis)
- Pain, muscle spasm and limitation of mobility
- Subcutaneous, intramuscular haematomas, psoas and retroperitoneal haematomas
- Gastrointestinal and genitourinary bleeding
- Splenomegaly (40% patients)

Laboratory Diagnosis

- Anaemia with neutrophilia
- Megakaryocytes are normal or increased in number
- PTT is prolonged; abnormal results of PTT only when Factor VIII C levels fall to <20–25% of normal.
- Biological or immunological assays are used to determine the levels of factor VIII. Factor VIII C level deficiency is classified as:
  - Severe deficiency: Factor VIII C level <0–2 U/dL
  - Moderate deficiency: Factor VIII C level 2–5 U/dL
  - Mild deficiency: Factor VIII C level >5 U/dL
- BT, CT abnormal

Q. Write briefly on von Willebrand disease.

Ans. von Willebrand disease is also called angiohaemophilia and pseudohaemophilia. It is second only to haemophilia A in frequency.

Genetics

- Classified into types I–III and a platelet type
- Types IIC and Type III are autosomal recessive
- Rest are autosomal dominant

Pathophysiology

- Abnormal or deficient VWF
- Synthesis of this macromolecule coded by a gene on chromosome 12
- VWF acts as a carrier of Factor VIII and is required for normal platelet adhesion

Laboratory Diagnosis

- **Prolonged BT** → mild bleeding, easy bruising, epistaxis and bleeding following minor procedures
- **Factor VIII C assay** → levels decreased
- **von Willebrand factor immunoassay** → levels decreased
- **Ristocetin-induced platelet aggregation delayed** → ristocetin induces platelet agglutination followed by secondary aggregation and release reaction; this process depends on binding of VWF to platelet membrane

Q. Write briefly on acquired coagulation disorders.

Ans. Acquired coagulation disorders may result from:

1. **Deficiency of vitamin K-dependent factors**
   (a) Haemorrhagic disease of newborn
   (b) Biliary obstruction
   (c) Malabsorption of vitamin K
   (d) Nutritional deficiency
   (e) Drugs:
      (i) Coumarins
      (ii) Broad spectrum antibiotics
      (iii) Cholestyramine
2. **Accelerated destruction of coagulation factors**
   (a) Disseminated intravascular coagulation (DIC)
   (b) Fibrinogenolysis (liver disease, thrombolytic agents, tumours postsurgery)
3. **Circulating inhibitors of coagulation**
   (a) Specific inhibitors
   (b) Lupus anticoagulant
   (c) Antithrombins
   (d) Paraproteins
4. **Miscellaneous causes**
   (a) After massive transfusion
   (b) After antibiotics and antineoplastic agents
   (c) Congenital heart disease, amyloidosis, nephrotic syndrome, Sheehan syndrome, Gaucher disease and leukaemias

Q. Write briefly on the aetiopathogenesis, clinical features and laboratory findings in disseminated intravascular coagulation (DIC).

Ans. DIC has the following clinico-haematological features:

Causes

- **Obstetric complications**
  - Abruptio placenta
  - Septic abortion/intrauterine death
  - Chorioamnionitis
  - Amniotic fluid embolism
  - Severe eclampsia
  - Degenerated H. mole and leiomyomas
  - Fetomaternal blood passage

- **Infections**
  - Viral: HSV, rubella, smallpox, hepatitis, CMV and epidemic haemorrhagic fever
  - Bacterial: Meningococcaemia and septicaemia (Gram-positive)
  - Mycotic: Histoplasmosis and aspergillosis
  - Protozoal: Malaria, kala-azar and trypanosomiasis
  - Metazoal: Heartworm disease in dogs
- **Neoplasms:** Carcinoma prostate, ovary, pancreas, breast, lung, carcinoid, rhabdomyosarcoma, neuroblastoma and acute promyelocytic leukaemia
- **Others:** PNH, incompatible transfusions, fresh water submersion and drug induced

Laboratory Diagnosis

1. **Acute DIC**

***Clinical findings*:**
- Multiple bleeding sites
- Ecchymoses of skin and mucous membranes
- Visceral haemorrhage

***Laboratory abnormalities*:** Consumption of clotting factors and platelets and intravascular haemolysis:
- Decreased levels of Factors II, V and VIII
- Fibrinogen level below 1.0 g/L
- Increased FDP (fibrin degradation products, eg, FDP, D dimer)
- Platelet count below 100×10^9 /L
- Prolonged thrombin time (deficiency of fibrinogen and increased FDP levels which inhibit thrombin activity), prolonged prothrombin time (PT) and activated partial thromboplastin time (PTTK)

2. **Chronic DIC**

***Clinical findings*:**
- Signs of deep venous or arterial thrombosis/embolism
- Superficial venous thrombosis

***Laboratory abnormalities*:**
- Modestly increased prothrombin time in some patients
- Shortened or lengthened partial thromboplastin time
- Normal thrombin time in most patients
- High, normal or low fibrinogen level
- High, normal or low platelet count
- Increased levels of FDP (eg, on testing for FDP, D dimer)

Q. What are blood groups? Enumerate the important blood group systems.

Ans. Blood groups are genetically determined antigens that can be detected on the RBC surface by specific antibodies (Table 12.22).

TABLE 12.22. Important blood group systems

Name of blood group system	Name of antigens
ABO	H, A_1, A_2 and B
Rh	D, C, E, c and e
P	P and p
MNS	M, N, S and s
Lutheran (Lu)	Lu^a and Lu^b
Lewis (Le)	Le^a and Le^b
Duffy (Fy)	Fy^a and Fy^b
Kidd (JK)	JK and JK

Q. What is the clinical significance of the ABO blood group system?

Ans. The ABO system (Table 12.23) is the product of one gene locus situated on chromosome 9, which determines the expression of ABO blood groups on RBCs, endothelial cells and some epithelial cells.

- The basic precursor substance in ABO antigens has a short chain of sugars.
- There are two types of chains; the Type I and Type II chains, which differ from each other in the way the terminal galactose joins the N-acetyl glucosamine residue.
- The basic precursor substance is converted to **H substance** by **L-fucosyltransferase** (**H gene** codes for this transferase that attaches fucose to the terminal end of the precursor substance to produce **H antigen**).
- The **A gene** codes for a transferase that attaches **N-acetylgalactosamine** to the precursor substance, thereby producing A antigen (blood group A).
- The **B gene** codes for a transferase that attaches **galactose** to the precursor substance to produce **B antigen** (blood group B).
- The **O gene** is inactive; hence, neither A, nor B antigens are present on the surface of blood group O RBCs.
- **Group AB** individuals have H antigen that carries both A or B active sugars.
- An individual receives one blood group antigen from the mother and one from the father.
- Antibodies belonging to ABO system are naturally occurring, IgM type, complete antibodies (capable of agglutinating RBCs in saline suspension).
- The A group contains about twenty subgroups, of which A1 and A2 are the most common. A1 makes up 80% of all A type blood while A2 makes up for the rest.

TABLE 12.23. ABO blood group

Blood group	Antigen on cell surface	Antibody in serum
A	A	Anti-A
B	B	Anti-B
AB	A and B	Neither
O	Neither anti-A or anti-B	Anti-A, B

Bombay Phenotype (hh)

Some individuals do not inherit the H gene and are not able to express substance H on their RBCs and thus, do not produce A or B antigens. Instead, they produce antibodies to substance H and both A and B antigens. They can receive blood only from other hh donors but can donate like group O individuals.

Routine ABO Grouping

- Includes both cell and serum testing
- ABO testing should be done at room temperature or lower
- Controls should always be run during ABO grouping

Two Types of Grouping

- Forward type identifies the blood group antigen on the surface of RBCs by using anti-A and anti-B test serum.
- In reverse grouping, group A and B red cells are allowed to react with patient serum to identify the isohaemagglutinins that correspond with the blood group.
- Before transfusion, the ABO system must be appropriately matched between recipient and donor.
- For example, a blood group A person, who has anti-B IgM antibodies, can receive only A or O blood.

- Individuals with blood group O can receive only O blood owing to the presence of anti-A IgM, anti-B IgM and anti-A,B IgG in their serum, which would destroy cells with A or B antigen on their surface.
- Individuals with blood group AB (universal recipient) may receive blood from any group, since they have no isohaemagglutinins to destroy the transfused cells.

Q. Write briefly on different blood group systems besides ABO system.

Ans. Blood group systems besides ABO system include

1. **Rh antigen system**
   (a) The Rh antigen system has three closely linked gene loci, coding for D antigen (there is no d antigen), C and/or c antigen and E and/or e antigen.
   (b) Thus, the antigens produced are C, D, E, c and e.
   (c) An individual may have similar or different sets of these three Rh antigens on each chromosome; for example, CDE/cde, cde/cde, or CdE/cdE (each person inherits one trio gene from each parent).
   (d) Individuals who are positive for D antigen are considered Rh-positive (85% of the population) and those who lack it are Rh-negative.
   (e) Individuals with a weak variant of D antigen, called the **Du variant,** are also considered Rh-positive.
   (f) **Alloimmunization** (formation of an antibody against an antigen) occurs if a person is exposed to an Rh antigen that is not on the patient's RBCs (eg, an Rh-negative person exposed to Rh-positive RBCs may develop anti-D antibodies).
   (g) The majority of clinically important antibodies that produce a transfusion reaction are warm-reacting (IgG) antibodies (eg, anti-D, anti-Kell) rather than cold-reacting (IgM) antibodies.
2. **Duffy antigen system:** African-Americans commonly lack **Duffy (Fy) antigens** on their RBCs, which protects their cells from *Plasmodium vivax* infestation, since *P. vivax* requires Duffy antigen as a receptor to bind to the RBCs.
3. **I antigen system:** May be associated with cold-reacting IgM antibodies against i antigen or I antigens leading to a cold autoimmune haemolytic anaemia (eg, anti-i is associated with infectious mononucleosis and anti-I with *Mycoplasma pneumoniae* infection).
4. **Lewis antigens** are closely related to ABH antigens and are produced in body secretions. Naturally occurring IgM antibodies develop against these antigens, but they are generally weak antibodies of no clinical importance.

Q. Write briefly on the principles of blood transfusion.

Ans. Blood transfusion involves the collection, storage and infusion of a donor's blood to a recipient. Routinely, ABO and Rh typing is done on donor blood, atypical antibodies, (eg, anti-D, anti-Kell) are tested for using the indirect Coombs test, serology is done for syphilis, hepatitis B, hepatitis C, HIV-1 and -2 and HTLV-1.

Indications for Blood Transfusion

- Traumatic haemorrhage
- Gynaecological blood loss
- Surgical blood loss
- Severe anaemias
- Coagulation disturbances
- Leukaemias
- Haemolytic anaemias
- Thalassaemia
- Peripheral circulatory failure/shock
- Burns

Blood Storage

- Plasma potassium, ammonia and phosphate increase during storage while RBC 2,3-bisphosphoglycerate (BPG) and plasma pH decrease during storage.
- The basic purpose of efficient blood storage is to increase the shelf life of preserved blood and to maintain high intra-erythrocyte BPG levels for the optimal oxygen exchange with tissue.
- **CPDA** (citrate–phosphate–dextrose–adenine) preserves cells for 35 days owing to the action of citrate as an anticoagulant, phosphate and adenine as substrates for ATP synthesis and dextrose as the source of anaerobic glycolysis in RBCs.

Crossmatch

The **standard pretransfusion tests** on the recipient consist of ABO group and Rh typing, an antibody screen for atypical antibodies (indirect Coombs test), a direct Coombs test (to identify IgG antibodies on RBCs) and a major crossmatch. The **major crossmatch** is accomplished in a test tube by mixing a sample of the recipient's serum with a sample of RBCs from the donor unit. The purpose of this crossmatch is to detect atypical (not naturally occurring) antibodies present in the recipient's serum that may be directed against foreign antigens on the RBCs in the donor unit.

Q. Write briefly on blood component therapy.

Ans. Different types of blood components:

- **Packed RBCs** are useful in the treatment of anaemia, since they have less volume and a higher haematocrit than whole blood. Each unit of packed RBCs should raise the Hb by 1 g/dL and the haematocrit by 3%.
- **Platelet transfusions** are generally indicated when patients have a platelet count $< 50{,}000$ cells/μL and have clinical evidence of bleeding or are candidates for a surgical or invasive procedure. Each unit of platelets infused should raise the platelet count by 5000–10,000 cells/μL.
- **Granulocyte transfusions** are reserved for patients who have severe sepsis associated with an absolute neutropenia < 500 cells/μL that has not responded appropriately to antibiotics within 48 h.
- **Fresh-frozen plasma** (FFP) contains all the coagulation factors and is the component of choice in bleeding associated with multiple factor deficiencies as seen in severe liver disease, Warfarin overdose and disseminated intravascular coagulation.
- **Cryoprecipitate** contains Factor VIII, fibrinogen, Factor XIII and fibronectin and is the component of choice in the treatment of mild von Willebrand disease and fibrinogen deficiency.
- **Factor VIII concentrates** are primarily used in the treatment of haemophilia A.
- **Albumin, plasma protein fraction** (PPF), **crystalloid solutions,** (eg, normal saline, Ringer lactate containing sodium, chloride, potassium, calcium and lactate) and **colloid substitutes,** eg, dextran and hydroxyethyl starch are utilized as volume expanders.
- **Immune serum globulin** is useful in the treatment of hypogammaglobulinaemia.

Q. What are transfusion reactions?

Ans. Transfusion reactions include

Immediate Reactions

- **Febrile reactions:** The most common early reactions and are due to the cytokines (IL1, IL6, IL8 and TNF) produced by leukocytes during storage. Characterized by fever, chills and headache, febrile reactions can be reduced in frequency by depletion of leukocytes.
- **Allergic reactions:** Flushing, urticaria, fever, tachycardia, wheezing, dyspnoea and cyanosis are secondary to antibodies directed against plasma proteins in the donor unit including IgA (Type I hypersensitivity reaction).

- **Acute haemolytic transfusion reactions:** Usually due to mismatched transfusion, these reactions are characterized by hypotension, heat and burning at the site of transfusion, fever, pain in the lower back or chest, bleeding due to DIC, oliguria due to renal failure. They may be intravascular or extravascular.
  - **Intravascular haemolytic transfusion reactions** are due to an **ABO mismatch** (type II hypersensitivity reaction).
  - **Extravascular haemolytic transfusion reactions** are due to presence of an atypical antibody in the recipient (eg, antiKell) that was undetectable (too low a titre) in the initial antibody screen.
- **Circulatory overload:** Rapid transfusion of blood may lead to congestive heart failure. Particularly prone are patients with severe anaemia and previous history of heart disease.
- **Endotoxic shock and fever due to bacterial contamination of blood.**

Delayed Transfusion Reactions

- **Delayed haemolytic transfusion:** Occur 3–10 days after the infusion of blood and are most often due to extravascular haemolysis from an atypical antibody reaction against donor RBCs
- **Transfusion hemosiderosis** due to chronic or multiple transfusions
- **Graft versus host reaction** due to granulocyte or WBC transfusion in immune-deficient patients

13

The Lung

AIRWAYS

Function: Exchange of gases between inspired air and blood

Histology: The entire respiratory tree is lined by pseudostratified tall columnar ciliated epithelium admixed with mucous-secreting goblet cells in the cartilaginous airways. Bronchial mucosa also has neuroendocrine cells that exhibit neurosecretory-type granules, which contain serotonin, calcitonin and gastrin-releasing peptide.

Structural hierarchy (Flowchart 13.1)

Bronchi
(have cartilaginous walls lined by columnar ciliated epithelium with mucous-producing submucosal glands)
↓ *Dichotomous branching*
Bronchioles
(lack cartilage and submucosal glands in their walls)
↓ *Further branching*
Terminal bronchioles
(bronchioles less than 2 mm in diameter)
↓
Acini
(spherical with a diameter of about 7 mm)

FLOWCHART 13.1. Structural hierarchy of airways.

Acinus

- ***An acinus has the following parts*** **(*Fig. 13.1*):**
  1. Respiratory bronchiole
  2. Alveolar duct
  3. Alveolar sac (blind end of respiratory passages and site for gas exchange)
- ***A cluster of 3–5 terminal bronchioles with its acinus is called a lobule.***

Alveolar wall

- ***The alveolar wall (alveolar septum; Fig. 13.2)*** is composed of the following layers:
  1. Capillary endothelium
  2. Basement membrane with surrounding interstitial tissue which separates the capillary endothelium from alveolar lining
  3. Alveolar epithelium, which is of two principal cell types:
     (a) Flattened Type I pneumocytes (cover 95% of alveolar surface)
     (b) Rounded Type II pneumocytes (are a source of pulmonary surfactant and participate in repair wherein they replace the damaged Type I pneumocytes)
- ***The alveolar macrophage*** is filled with carbon and lying loose in the alveolar spaces.

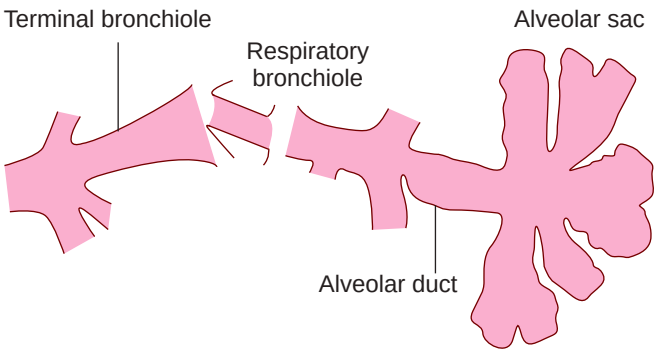


FIGURE 13.1. Parts of an acinus.

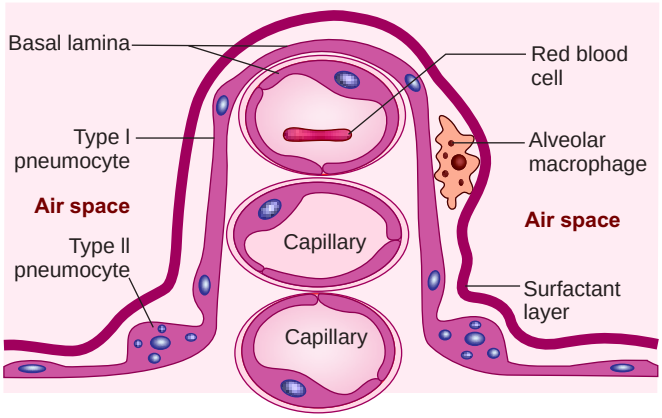


FIGURE 13.2. Schematic diagram of the structure of the alveolar septum.

Q. Write briefly on acute respiratory distress syndrome (ARDS).

Ans. ARDS is a manifestation of severe acute lung injury or ALI.

Salient Features

- Also known as diffuse alveolar damage, and shock lung
- Caused by **diffuse alveolar capillary damage**
- Presents with rapid onset of severe life-threatening respiratory insufficiency, cyanosis and severe arterial hypoxaemia in the absence of cardiac failure. The respiratory insufficiency may be refractory to O2 therapy.
- May progress to extra pulmonary multisystem organ failure

Causes

- Infections
  - Sepsis
  - Diffuse pulmonary infections (viral, mycoplasma, pneumocystis pneumonia and miliary tuberculosis)
- Physical injuries
  - Head injury
  - Pulmonary contusions
  - Drowning
  - Fractures and fat embolism

  - Burns
  - Ionizing radiation
- Pulmonary embolization
- Inhalation of irritants
  - Oxygen toxicity
  - Smoke
  - Gases and chemicals like ammonia, chlorine and nitrogen dioxide
- Chemical injury
  - Heroin or methadone or barbiturate overdose
  - Acetylsalicylic acid
  - Thiazides
- Haematological conditions
  - Multiple transfusions
  - DIC
- Others
  - Pancreatitis
  - Uraemia
  - Cardiopulmonary pass
  - Hypersensitivity reactions

Gross Pathology

Heavy, red, boggy lungs, which ooze fluid on cutting

Microscopy

- **A**lveolar lining and pulmonary capillary endothelium are damaged.
- Alveolar walls are lined by a **waxy hyaline membrane** consisting of fibrin-rich oedema fluid mixed with cytoplasmic and lipid remnants of necrotic epithelial cells.
- Type II pneumocytes proliferate to regenerate alveolar lining.
- **F**ibrin exudates organize and intra-alveolar fibrosis may ensue.
- **Resolution is unusual; ARDS is commonly fatal.**

X-ray

Shows diffuse bilateral infiltrates

Q. Define atelectasis. Enumerate and describe its various types.

Ans. Definition: Incomplete expansion of the lungs at birth (neonatal atelectasis) or collapse of previously inflated lungs produces areas of relatively airless parenchyma.

Types:

1. **Resorption atelectasis** (Flowchart 13.2)
2. **Compression atelectasis** (Flowchart 13.3)
3. **Contraction atelectasis:**

Mucous plugs and exudates in smaller bronchi (seen in asthma, bronchitis, bronchiectasis, post-operative states) and aspiration of foreign bodies.

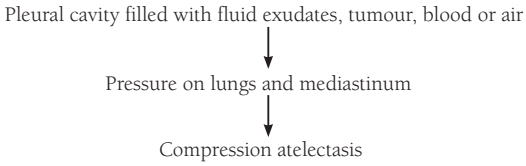
↓

Complete obstruction of airway

↓

Resorption of air trapped in dependent alveoli, leading to resorption atelectasis

FLOWCHART 13.2. Mechanism of development of resorption atelectasis.



FLOWCHART 13.3. Mechanism of development of compression atelectasis.

Occurs due to localized or generalized fibrosis of lungs or pleura preventing their full expansion.

Q. Write briefly on chronic obstructive pulmonary disease (COPD).

Ans. COPD occurs as a result of partial or complete chronic obstruction of airflow. Disorders associated with airflow obstruction include

1. Emphysema
2. Chronic bronchitis
3. Asthma
4. Bronchiectasis
5. Small airway disease (bronchiolitis)

Emphysema

Definition

Abnormal permanent enlargement of the air spaces distal to terminal bronchiole (including respiratory bronchiole, alveolar duct and alveolus), accompanied by destruction of their walls without fibrosis. Dilatation of air spaces without destruction is called **overinflation.**

Types

Emphysema is classified according to the anatomic distribution into four major types (Fig. 13.3):

1. **Centriacinar (centrilobular) emphysema**
   (a) Affects central or proximal part of acini and spares the distal part (in severe centriacinar emphysema, distal acinus may be involved, making it panacinar)
   (b) More common in upper lobes
   (c) Predominantly seen in heavy smokers in association with chronic bronchitis
   (d) May coexist with coal worker's pneumoconiosis (walls of emphysematous spaces demonstrate large amounts of pigment)
   (e) Peribronchial and bronchiolar spaces commonly show inflammation
2. **Panacinar (panlobular) emphysema**
   (a) Acini are uniformly enlarged from the level of respiratory bronchiole to terminal blind alveolar sac
   (b) More common in lower zones and anterior margin of lungs. Most severe at the bases
   (c) Associated with α-1 antitrypsin (α-1 AT) deficiency
3. **Paraseptal (distal acinar)**
   (a) Distal part of acinus is involved; proximal part is normal
   (b) Localized along pleura and perilobular septae
   (c) Usually seen in upper part of lungs; adjacent to areas of fibrosis and atelectasis
   (d) Spontaneous pneumothorax is a common complication
   (e) Characteristic finding is presence of multiple, continuous and enlarged airspaces 0.5–2 cm, forming cyst-like structures.
4. **Irregular (paracicatricial) emphysema**
   (a) Irregular involvement of acinus
   (b) **Usually asymptomatic and clinically insignificant**

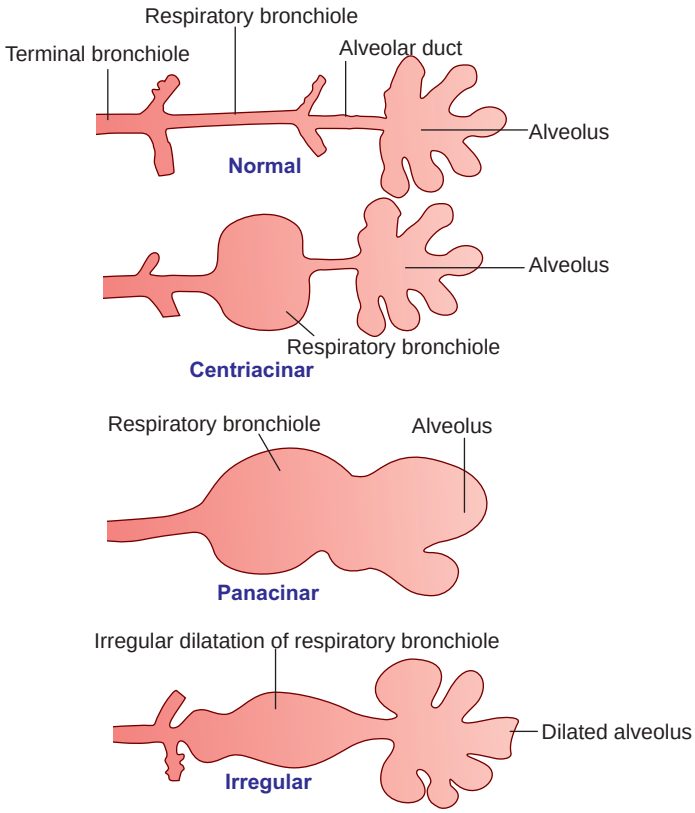


FIGURE 13.3. Types of emphysema.

Others Types of Emphysema

Sometimes the term 'emphysema' may be applied to certain conditions not conforming strictly to the definition of emphysema, eg,

1. **Compensatory emphysema:** Dilatation of alveoli without destruction of septal walls in response to loss/removal of lung substance elsewhere
2. **Obstructive overinflation:** Expansion of lung because of air trapped within it. Trapping of air may be due to
   (a) Subtotal obstruction: Air enters in inspiration but cannot exit in expiration (ball valve mechanism)
   (b) Total obstruction: Ventilation through collaterals (pores of Kohn and canals of Lambert), which bring in air behind the obstruction
3. **Bullous emphysema:**
   (a) A form of emphysema that produces large subpleural bullae (blebs $>$ 1 cm in size), rupture of which may lead to pneumothorax
   (b) Usually a consequence of old tuberculous scarring
4. **Interstitial emphysema**
   (a) Characterized by air entry into connective tissue stroma of lung, mediastinum or subcutaneous tissue
   (b) Causes include an alveolar tear caused by bronchiolar obstruction accompanied by explosive coughing (whooping cough, bronchitis, etc.) or puncture of the lung due to a chest wound or a fractured rib

Pathogenesis of Emphysema

- **Role of 'Protease–antiprotease' mechanism:**
  - Homozygous patients with a genetic deficiency of protease inhibitor α-1 AT have an increased tendency to develop emphysema. Smoking exaggerates this tendency.

- α-1 AT is synthesized in liver and is present in serum, tissue fluid and macrophages.
- Normal α-1 AT phenotype is PiMM.
- α-1 AT deficient phenotype is PiZZ.
- Eighty percent PiZZ patients end up with emphysema.
- Pi null phenotype has no detectable levels of α1 AT.

- ***Role of neutrophils*:** Neutrophils are a source of:
  - Elastase activity
  - Cellular proteases (proteinase 3 and cathepsin)
  - Matrix metalloproteinases
  - Oxygen-derived free radicals (inactivate native antiproteases by oxidative injury)
- ***Role of smoking*:** Smoking enhances elastase activity of macrophages. Macrophage elastase is not inhibited by α-1 AT and it can, in fact, digest the latter.

Clinical Features of Emphysema

Patients do not become symptomatic until at least one-third of functional parenchyma is damaged. Presenting signs and symptoms of emphysema include

- Dyspnoea
- Cough (late and with scanty sputum)
- Severe weight loss
- Barrel-shaped chest
- Prolonged expiration (key to diagnosis)
- Hunched position and breathing through pursed lips
- Death may be due to either of the following:
  - Respiratory acidosis and coma
  - Right-sided heart failure
  - Massive collapse of lungs secondary to pneumothorax

Chronic Bronchitis

Definition

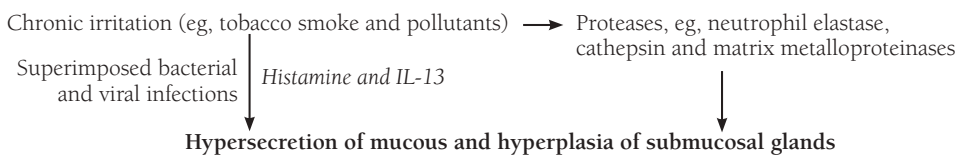
Persistent cough with sputum production for at least three months in two consecutive years. It is **common in habitual smokers and inhabitants of smoke-laden cities and may progress to**

- COPD
- Cor pulmonale and heart failure
- Atypical metaplasia and dysplasia of respiratory epithelium

Types

1. **Simple chronic bronchitis:** Productive cough but no physiologic evidence of airflow obstruction
2. **Chronic asthmatic bronchitis:** Hyperactive airways with intermittent bronchospasm and wheezing
3. **Obstructive chronic bronchitis:** Some patients, eg, heavy smokers, develop chronic airflow obstruction usually with associated emphysema

Pathogenesis (Flowchart 13.4)



FLOWCHART 13.4. Pathogenesis of chronic bronchitis.

Cigarette smoking predisposes to infection by

- Interfering with ciliary action of respiratory epithelium and function of alveolar macrophages
- Causing direct damage to airway epithelium
- Leading to hypertrophy and hyperplasia of mucous glands
- Inhibiting ability of bronchial and alveolar leukocytes to clear bacteria

Gross Morphology

- Hyperaemia, swelling and oedema of airways
- Excessive mucinous to mucopurulent secretions in the bronchi and bronchioles

Microscopy (Fig. 13.4)

- Chronic (predominantly lymphocytic) inflammation of airways
- Enlargement of mucous-secreting glands of trachea and bronchi
- Increase in number of goblet cells and mucous glands in airways
- Increase in **Reid index** (ratio of thickness of the mucous gland layer to the thickness of the wall between the epithelium and the cartilage (normal 0.4))
- Squamous metaplasia and dysplasia
- Obliteration of lumina of bronchioles due to fibrosis (bronchiolitis obliterans)

Histopathological changes in small airways in young smokers include

- Goblet cell metaplasia with mucous plugging
- Clustering of pigmented alveolar macrophages and infiltration by inflammatory cells
- Fibrosis of bronchiolar wall

Clinical Features

- Persistent cough with production of sputum
- Later stages, hypercapnia, hypoxaemia and mild cyanosis
- Death is due to severe infection or cor pulmonale and cardiac failure

Bronchial Asthma

Definition

Asthma is a chronic inflammatory disorder of the airways that causes recurrent episodes of wheezing, breathlessness, chest discomfort and cough particularly at night and/early

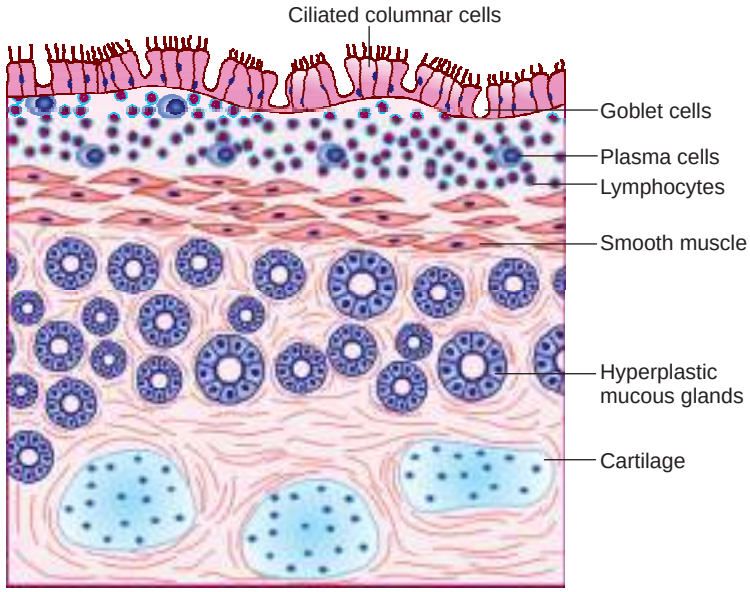


FIGURE 13.4. Histopathology of chronic bronchitis showing chronic inflammation of airways, enlargement of mucous-secreting glands of trachea and bronchi, increase in number of goblet cells and mucous glands in airways and increase in Reid index.

morning. It is associated with variable degree of bronchoconstriction, inflammation of the bronchial walls and increased mucous secretion.

Pathogenesis

- **Genetic predisposition to Type I hypersensitivity (atopy)** and **exposure to certain environmental triggers (inhaled allergens** like house dust, mites, pets, etc., **viruses** like rhinovirus and respiratory syncytial virus; **air pollutants, smoking** and **drugs** like beta adrenergic blockers and aspirin) induce bronchial hyper-responsiveness leading to acute and chronic airway inflammation.
- **T_H2 cells** induce bronchial inflammation and secrete cytokines like interleukin-4 (which stimulates B cells to produce IgE); interleukin-5 (which activates eosinophils) and interleukin-13 (which stimulates mucous secretion from bronchial submucous glands)
- **T_H1 cells normally secrete cytokines that inhibit T_H2 cells and vice versa.** Imbalance in this reciprocal arrangement can lead to the development of asthma. T_H1 cells produce **γ** interferon, which suppresses inflammation in airways. A transcription factor, called **T-bet,** required for **T_H1** cell differentiation, is found to be absent from lung lymphocytes in asthmatic patients. In the absence of the restraining influence of interferon **γ**, **T_H2** cells provoke airway inflammation.
- Microenvironment in the bronchial wall may be altered due to **ADAM 33 polymorphisms** (ADAM 33 is expressed by lung fibroblasts and bronchial smooth muscle cells and belongs to a subfamily of matrix metalloproteinases; Flowchart 13.5)

ADAM polymorphisms
↓ *Unknown mechanism*
Accelerated proliferation of bronchial smooth muscle cells and fibroblasts
↓ • *Recruitment of mast cells*
• *Release of vasoactive mediators, cytokines and growth factors*
Structural changes in bronchial wall **(airway remodelling)**

FLOWCHART 13.5. Airway remodelling due to ADAM polymorphisms.

Types

1. Based on frequency and severity of symptoms
   (a) Mild intermittent
   (b) Mild
   (c) Moderate
   (d) Severe persistent
2. Based on response to steroids
   (a) Steroid dependent
   (b) Steroid resistant
3. Based on initiating factors
   (a) Extrinsic: Induced by an extrinsic antigen and initiated by a Type 1 hypersensitivity reaction
   (b) Intrinsic: Initiated by diverse nonimmune mechanisms, eg, aspirin ingestion, cold, stress, exercise, precipitated by several factors unique to the patient
4. Based on aetiology
   (a) Atopic asthma (Type 1 IgE-mediated response triggered by environmental allergens like food, dust, pollen, animal dander; most common type of asthma; begins in childhood; positive family history and skin reaction)
   (b) Nonatopic asthma (No identifiable causative external agents; no family history or positive skin test)

Gross Morphology

- Overinflated lungs with small areas of atelectasis
- Occlusion of bronchi and bronchioles by thick tenacious mucous plugs

Microscopy

- Thickening of basement membrane of bronchial epithelium
- Oedema and inflammation in the bronchial walls (cells involved are eosinophils and mast cells)
- Presence of **Charcot–Leyden crystals** (crystalloids made of eosinophil membrane protein)
- Mucous plugs in bronchi and bronchioles containing whorls of shed epithelium (**Curschmann spirals**)
- Increase in size of submucosal glands and hypertrophy of bronchial wall muscle

Clinical Features

- During an asthmatic episode the patient presents with chest tightness, dyspnoea, wheezing and cough without production of sputum.
- Patient may be asymptomatic between asthmatic episodes.
- The most severe form of asthma is labelled 'status asthmaticus'. This may last for days to weeks, be unresponsive to treatment and lead to severe cyanosis and sometimes death.

Bronchiectasis

Definition

Abnormal and permanent dilatation of proximal and medium-sized bronchi (> 2 mm in diameter), caused by destruction of the muscular and elastic components of the bronchial walls.

Causes

- **Congenital bronchiectasis** results from developmental arrest of the bronchial tree, eg, bronchopulmonary sequestration, which is classified as either intralobar or extralobar and results in chronic lower respiratory tact infections that later lead to bronchiectasis.
- The **more common acquired forms** occur in adults and older children and require an infectious insult, impairment of drainage, airway obstruction and/or a defect in host defence. May be due to:
  - **Primary infections:** Bronchiectasis may result as a consequence of necrotizing infections that are either poorly treated or not treated at all. Typical offending organisms include *Klebsiella* species, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, *Mycobacterium avium* complex and certain viruses.
  - **Bronchial obstruction:** Endobronchial tumours, foreign body impaction, right middle lobe syndrome (results from an abnormal angulation of the lobar bronchus at its origin, predisposing it to obstruction)
  - **Cystic fibrosis (CF):** Bronchiectasis associated with CF is secondary to mucous plugging of proximal airways and chronic pulmonary infection, especially with *P. aeruginosa*.
  - **Young syndrome:** This genetic variant of CF presenting with bronchiectasis, sinusitis and azoospermia.
  - **Primary ciliary dyskinesia:** It presents with immotile or dyskinetic cilia and/or sperms. This may lead to poor mucociliary clearance, recurrent pulmonary infections and ultimately, bronchiectasis. A variant of this condition, initially described by **Kartagener**, comprises the clinical triad of situs inversus, nasal polyps, sinusitis and bronchiectasis due to immotile cilia of the respiratory tract.

Gross Morphology

- Bronchiectasis may present as: (1) a focal process involving a lobe or a segment of the lung or (2) a diffuse process involving both lungs. The former is the most common

presentation of bronchiectasis, while the latter is most often associated with systemic illnesses.

- The bronchi and bronchioles are dilated and can be traced up to the pleural surface.
- The wall of the bronchi is thickened due to fibrosis and the lumen may be filled with mucopurulent secretions.
- Reid characterized bronchiectasis as cylindrical, cystic or varicose based on morphology:
  - *Cylindrical bronchiectasis*: Bronchi are dilated minimally and have straight, regular outlines (primarily due to mucosal oedema)
  - *Cystic or saccular bronchiectasis*: Bronchi have a ballooned appearance and demonstrate air-fluid levels (due to ulceration with bronchial neovascularization).
  - *Varicose bronchiectasis*: Bulbous bronchi with dilatations and intervening sites of relative constriction due to scarring.

Microscopy

In an active case, there may be acute and chronic inflammation of the bronchi and bronchioles, desquamation of the epithelium and necrotizing ulceration. In more chronic cases, fibrosis of the bronchial and bronchiolar walls is seen (Fig. 13.5).

Clinical Presentation

- Cough with mucopurulent, often foul smelling sputum, lasting months to years
- Haemoptysis may result from airway damage associated with acute infection.
- Less specific symptoms include dyspnoea, pleuritic chest pain, wheezing, fever, weakness and weight loss

Signs

Pallor, cyanosis, abnormal chest sounds, foul smelling breath and digital clubbing

Complications

1. Recurrent pneumonias
2. Lung abscess
3. Respiratory failure
4. Cor pulmonale
5. Empyema
6. Amyloidosis

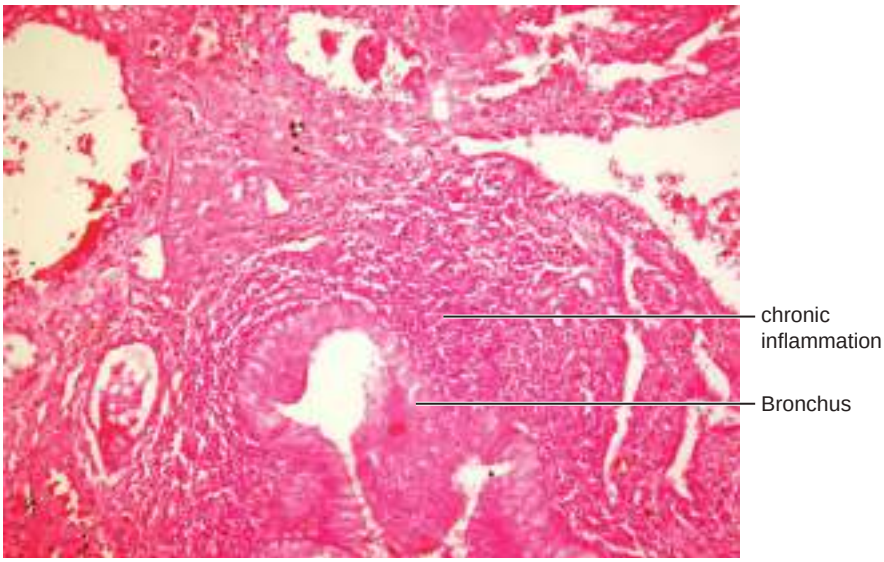


FIGURE 13.5. Section showing chronic inflammation of the bronchi and bronchioles (H&E; 200×).

Q. Differentiate between centriacinar and panacinar emphysema.

Ans. Differences between centriacinar and panacinar emphysema are shown in Table 13.1.

TABLE 13.1. Differences between centriacinar and panacinar emphysema

Features	Centriacinar emphysema	Panacinar emphysema
Distribution	Central or proximal part of acini affected and distal acini spared	Acini uniformly enlarged from the level of respiratory bronchiole to terminal blind alveolar sac
Part of lung involved	More common in upper lobes	More common in lower zones and anterior margin of lungs. Most severe at the bases
Aetiology	Predominantly seen in heavy smokers, often in association with chronic bronchitis	Associated with α-1 AT deficiency
Pigment deposition	Walls of emphysematous spaces contain large amounts of pigment (associated with coal worker's pneumoconiosis)	No such findings or association
Inflammation	Inflammation around bronchi and bronchioles is commonly encountered	Not seen
Frequency of occurrence	More common	Less common

Q. Differentiate between chronic bronchitis and emphysema.

Ans. Differences between chronic bronchitis and emphysema are shown in Table 13.2.

TABLE 13.2.

Features	Chronic bronchitis	Emphysema
Age	40–45 years	50–75 years
Dyspnoea	Mild and late	Severe and early
Cough	Early onset with copious sputum	Late onset with scanty sputum
Infections	Common	Occasional
Respiratory insufficiency	Repeated	Terminal
Cor pulmonale	Common	Rare and terminal
Airway resistance	Increased	Normal or slightly increased
X-ray chest	Large heart	Small heart
Physical appearance	Blue bloater	Pink puffer

Q. Differentiate between extrinsic and intrinsic asthma.

Ans. Differences between extrinsic and intrinsic asthma are shown in Table 13.3.

TABLE 13.3. Differences between extrinsic and intrinsic asthma

Features	Extrinsic asthma	Intrinsic asthma
Evolution	Induced by a Type I hypersensitivity reaction due to an extrinsic antigen; positive skin hypersensitivity tests	Immune mechanism not involved; precipitated by some variable idiosyncratic reactions; negative skin hypersensitivity tests
Family history	Present	Absent
Age group affected	Childhood	Adults
Other allergies, eg, rhinitis, urticaria, eczema	Present	Absent
Serum IgE	Increased	Normal
Associated emphysema	Rare	Common
Associated bronchitis	Absent	Present

Q. Enumerate the various pulmonary infections.

Ans. Common pulmonary infections include

1. Acute pneumonia
2. Health care–associated pneumonia
3. Hospital-acquired pneumonia
4. Aspiration (inhalation) pneumonia
5. Chronic pneumonia
6. Pneumonia in an immunocompromised host
7. Necrotizing pneumonia and lung abscess

Note: For details on pneumonia, see Chapter 7.

Q. Enumerate the complications of acute bacterial pneumonia.

Ans. Complications of acute bacterial pneumonia:

1. **Abscess formation:** Due to tissue destruction and necrosis (more in case of *Klebsiella* or Type III pneumococcal infections)
2. **Empyema:** Presence of suppurative material in the pleural cavity
3. **Organization of intra-alveolar exudate may convert affected lung into solid fibrous tissue.**
4. **Bacteraemia dissemination:** Heart valves (endocarditis), pericardium (pericarditis), brain (meningitis), joints (suppurative arthritis) and metastatic abscesses in kidneys, spleen, etc.

Q. Write briefly on community-acquired acute viral pneumonia.

Ans. Community-acquired acute viral pneumonia has the following clinicopathological features:

Causative Organisms

Respiratory syncytial virus, parainfluenza virus, human metapneumovirus, influenza A and B and adenovirus

Predisposing Conditions

Malnutrition, alcohol intake and diminished immunity

Clinical Features

- Nonspecific
- May **mimic an upper respiratory tract infection or present as an acute nonspecific febrile illness manifesting with fever, headache and myalgias in immunocompetent individuals**
- May present as a **life-threatening infection in immunocompromised individuals**

Gross Morphology

- Involvement patchy or lobar
- May be unilateral or bilateral
- Lungs are red-blue, congested, subcrepitant; pleural involvement is rare

Microscopy

- Inflammation is restricted to the alveolar walls; alveolar space is free from exudate (thus called atypical pneumonia). In contrast, in bacterial pneumonia the exudate is typically intra-alveolar.
- The inflammatory infiltrate is composed of lymphocytes, histiocytes and plasma cells.
- Intra-alveolar spaces show proteinaceous material and the alveolar septal walls are lined by pink hyaline membrane.
- Superimposed bacterial infections may lead to a picture-like bacterial pneumonia.
- In **cytomegalovirus infection**, giant cells with intranuclear or intracytoplasmic inclusions may be seen.

Q. Outline the aetiopathogenesis, morphology and complications of lung abscess.

Ans. Lung abscess is a localized suppurative process within the lungs which induces necrosis of lung tissue.

Aetiopathogenesis

Causative Organisms

- *Aerobic* and *anaerobic streptococci*
- *Staphylococcus aureus*
- *Bacteroides*
- *Fusobacterium*

Predisposing Factors

- Oropharyngeal: Surgical procedures, dental sepsis and sinusitis
- Bronchial obstruction: Secretions, bronchiectasis and bronchogenic carcinoma
- Direct trauma, infection from other organs (direct and haematogenous spread)
- Aspiration of infective material: Gastric contents may be aspirated in comatose patients, alcoholics, patients under anaesthesia, or with sinusitis and depressed cough reflex
- Antecedent primary bacterial infection particularly in immunosuppressed patients (*Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pyogenes* and *Pseudomonas*)
- Septic embolism: From thrombophlebitis (affecting systemic veins) or infective bacterial endocarditis vegetations in right side of the heart
- Idiopathic/primary cryptogenic lung abscess: No definite cause is found.

Morphology

- Characterized by suppurative destruction of the lung parenchyma with a central area of cavitation
- May be solitary or multiple
- Pulmonary abscesses due to aspiration are more common on the right side due to the more vertical right main bronchus; abscesses that develop secondary to pneumonias and bronchiectasis are usually multiple, basal and diffusely scattered
- Abscesses due to septic emboli and pyemia are also multiple but may affect any region of the lungs
- Superimposed saprophytic infections lead to a large, ill-defined, foul-smelling and multilocular cavity **(gangrene of the lung).**

Complications

- Involvement of pleural cavity by extension of the infection (empyema)
- Haemorrhage

- Metastatic brain abscess or meningitis from septic emboli
- Secondary amyloidosis
- Clubbing of the fingers and toes

Q. Write in detail on the aetiopathogenesis, morphological (or autopsy) findings, sequelae and complications of pulmonary tuberculosis.

Ans. See Chapter 7.

Q. Classify chronic interstitial lung diseases (ILD). Write briefly on their clinicopathological features.

Ans. Chronic interstitial lung diseases are a heterogeneous group of disorders characterized by the diffuse and chronic involvement of pulmonary connective tissue, principally the alveolar interstitium.

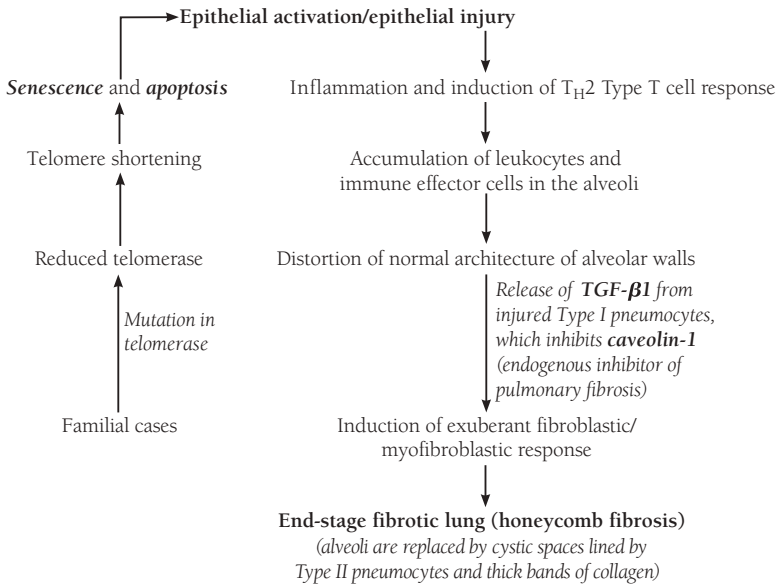
Classification

1. Fibrosing alveolitis
   (a) Usual interstitial pneumonitis (UIP) or idiopathic pulmonary fibrosis
   (b) Nonspecific interstitial pneumonia (NSIP)
   (c) Cryptogenic organizing pneumonia (COP)
   (d) Interstitial lung disease associated with
      (i) Collagen vascular diseases
      (ii) Pneumoconiosis
      (iii) Drug reactions
      (iv) Radiation injury
2. Granulomatous ILD
   (a) Sarcoidosis
   (b) Hypersensitivity pneumonitis
3. Eosinophilic ILD
4. Smoking-related ILD
   (a) Desquamating interstitial pneumonia (DIP)
   (b) Respiratory bronchiolitis-associated interstitial lung disease
5. Pulmonary alveolar proteinosis

Salient Features

- The aetiopathogenesis of many of the above conditions is unknown or not clearly understood.
- Patients of ILD usually present with dyspnoea, tachypnoea, end-inspiratory crackles and cyanosis without wheezing (clinical and pulmonary functional changes are those of restrictive rather than obstructive nature).
- Classical physiologic features are decreased carbon monoxide diffusing capacity and reduced lung volume and compliance.
- X-ray chest shows diffuse infiltration by small nodules, irregular lines or ground glass shadows.
- Eventually, patient goes into secondary pulmonary hypertension and cor pulmonale.
- Scarring or gross destruction of lung leads to end stage or honeycomb lung.

Pathogenesis (Flowchart 13.6)



FLOWCHART 13.6. Pathogenesis of ILD.

Q. Write briefly on idiopathic pulmonary fibrosis (IPF).

Ans. IPF is characterized by the following salient features:

- Causes progressive interstitial fibrosis leading to lung failure
- Usually affects individuals older than 50 years
- Histological picture labelled as UIP but can have overlapping features with other entities (connective tissue disorders, hypersensitivity pneumonia and asbestosis). Diagnosis should be based on the complete clinics-radiologic picture and morphology.

Pathogenesis

Both genetic and environmental factors implicated.

1. **Environmental factors:** Smoking, exposure to toxins such as metal fumes, occupational exposure (farming, stone polishing and hair dressing) damages alveolar epithelium activates immune responses to generate profibrogenic factors resulting in collagen production and fibrosis.
2. **Genetic factors:** Telomerase mutations, surfactant mutations and genetic abnormality leading to increased secretion of MUC5B, an abnormal mucin that predisposes to alveolar epithelial injury.

Histopathology

- Prominent fibrosis along the interlobular septae and subpleural region with patchy interstitial fibrosis.
- Early lesions are cellular, contain plump fibroblasts and late lesions are densely collagenized.
- Fibrosis induces distortion of cellular architecture and formation of cystic spaces resulting in a 'honeycoomb appearance'.
- Chronic inflammation may be seen in the areas of fibrosis.

Clinical Features

- Insidious onset with progressively increasing dyspnoea and dry cough.
- Hypoxaemia, clubbing and cyanosis are seen in later stages.
- The disease course is variable; mostly the patient worsens despite immunosuppressants.

Q. Write briefly on cryptogenic organizing pneumonia.

Ans. ***Salient features of cryptogenic organizing pneumonia***

- A disease of unknown aetiology in which patient presents with cough and dyspnoea
- X-ray shows patchy peribronchial and subpleural consolidation
- Microscopy shows polypoid bits of loose connective tissue in the bronchioles, alveoli and alveolar ducts (**Masson bodies**). No interstitial fibrosis is seen and most patients recover after being given steroids for 6 months or longer.

Q. Write briefly on pulmonary involvement in autoimmune diseases.

Ans. Lungs can be involved in the following autoimmune diseases:

1. **Rheumatoid arthritis**
   - Involved in 30–40% patients
   - May present as chronic pleuritis, pleural effusion, interstitial pneumonitis or fibrosis and follicular bronchiolitis
2. **Scleroderma or systemic sclerosis**
   Diffuse interstitial fibrosis and pleural involvement
3. **SLE**
   Transient infiltrates, pneumonitis and pleural involvement

Q. Write in detail on the clinicopathological features of sarcoidosis.

Ans. Sarcoidosis is a systemic disease of unknown cause characterized by formation of noncaseating granulomas in different tissues and organs.

Aetiopathogenesis

Unknown; but consensus is that disordered immune regulation, genetic predisposition and presence of certain environmental agents may all contribute.

Immunological Abnormalities

- Intra-alveolar and interstitial accumulation of CD4+ T cells leads to increased CD4:CD8 T cells ratio.
- Increased levels of T-cell-derived T_H1 cytokines such as IL2 and IFN γ, results in T cell expansion and macrophage activation.
- Increased levels of other cytokines like IL-8, TNF, macrophage inflammatory protein 1α (MIP1 α), which recruit additional T cells and monocytes and contribute to the formation of granulomas.
- Anergy to *candida* and common skin antigens like purified protein derivative (PPD) and polyclonal hypergammaglobulinaemia may be seen.

Genetic Association

Familial clustering of cases and association with certain HLA haplotypes (HLA-A1 and HLA-B8)

Environmental Contribution

Several microbes have been implicated in the pathogenesis of sarcoidosis, eg, *Mycobacteria*, *Propionibacterium acnes, Borrelia burgdorferi, viruses, fungi* and *Rickettsia* species.

Morphology

- Involved tissues show noncaseating granulomas composed of closely packed epithelioid cells with Langhans or foreign body giant cells (lymphocytes are few in number, so granulomas also called 'naked granulomas').
- Long-standing granulomas are enclosed within fibrous rims or hyaline scars and may show the following inclusions:
  - Laminated concretions composed of calcium and proteins known as **Schaumann bodies**
  - Stellate inclusions in giant cells called **asteroid bodies**

1. **Lungs**
   (a) Most commonly involved; show granulomas which coalesce to produce small palpable nodules around lymphatics, bronchi, blood vessels and sometimes within alveoli; heal with fibrosis
   (c) Pleural surfaces may sometimes be involved
2. **Lymph nodes**
   (a) Involved in almost all cases; sarcoidosis mainly affects hilar and mediastinal nodes, may occasionally manifest as generalized lymphadenopathy
   (b) Nodes are enlarged, discrete, nontender and sometimes calcified.
   (c) Tonsils may also be involved in some cases.
3. **Spleen**
   (a) Microscopic involvement of spleen is seen in three-fourth cases but gross enlargement is seen in very few cases
   (b) Contains scattered granulomas
4. **Liver**
   (a) Involved less often than spleen
   (b) Shows scattered granulomas located more often in the portal triads than the lobular parenchyma
5. **Bone marrow**
   (a) Typically involves the phalangeal bones creating small-circumscribed areas of lysis.
   (b) Widening of bony shafts and new bone formation on the outer surfaces may be seen.
6. **Skin and mucosa**
   (a) Skin lesions are encountered in about 50% cases.
   (b) Include discrete subcutaneous nodules; erythematous plaques or red scaly flat lesions.
   (c) Lesions may also appear in the mucous membranes of oral cavity, larynx and upper respiratory tract.
7. **Eye**
   (a) May cause iritis or iridocyclitis, corneal opacities, glaucoma and total loss of vision.
   (b) Inflammation of lacrimal glands and suppression of lacrimation are commonly encountered.
8. **Salivary glands**
   (a) Bilateral involvement of the salivary glands is usual.
   (b) Combined uveoparotid involvement is labelled **Mikulicz syndrome.**
9. **Muscle:** Involvement of muscle manifests as muscle weakness, tenderness and fatigue.

Sarcoid granulomas may also be seen in CNS, endocrine organs, kidneys and heart.

Clinical Features

- Usually discovered accidentally on routine X-ray or CT scan
- Insidious onset of respiratory symptoms (shortness of breath, cough, chest pain and haemoptysis)

- Constitutional signs and symptoms include fever, fatigue, weight loss, anorexia and night sweats
- May present with peripheral lymphadenopathy, cutaneous lesions, eye involvement or hepatosplenomegaly
- Chronic progressive course or alternating periods of remission (spontaneous or steroid induced) and activity is typical
- Most resolve with minimal or no residual manifestations
- Most succumb to progressive pulmonary fibrosis and cor pulmonale

Q. Write briefly on hypersensitive pneumonitis.

Ans. Hypersensitivity (allergic) pneumonitis is an immunologically mediated response to an extrinsic antigen involving, initially immune complex (Type III) and later granulomatous (Type IV hypersensitivity). It includes

- **Farmer lung** caused by moldy hay containing actinomycetes (external antigen)
- **Pigeon breeder's disease** or **bird fancier's disease** caused by proteins from serum, excreta or feathers of birds
- **Humidifier** or **air-conditioner lung** caused by thermophilic bacteria in heated water reservoirs

Clinical Features

- Acute form: Recurring episodes of fever, dyspnoea, cough and leukocytosis
- Chronic form: Signs of progressive respiratory failure, dyspnoea, cyanosis and reduced lung compliance

X-Ray

Diffuse or nodular infiltrates

Pulmonary Function Tests

These are indicative of a restrictive disorder

Morphology

Histological changes are mainly seen in bronchioles and include

- Interstitial pneumonitis consisting of lymphocytes, plasma cells and macrophages
- Noncaseating granulomas
- Interstitial fibrosis and obliterative bronchiolitis (in late stage)

Q. Write briefly on pulmonary eosinophilia.

Ans. Pulmonary eosinophilia includes several entities, eg, acute eosinophilic pneumonia with respiratory failure, secondary eosinophilia and idiopathic chronic eosinophilic pneumonia.

1. ***Acute eosinophilic pneumonia with respiratory failure*:** It is a steroid-responsive disease which presents with hypoxaemia, fever, dyspnoea and pulmonary infiltrates. The bronchoalveolar lavage fluid contains more than 25% eosinophils and histopathology shows extensive alveolar damage with eosinophilic infiltration.
2. ***Secondary eosinophilia*:** Occurs secondary to bacterial, fungal and parasitic infections, hypersensitivity pneumonitis, drug allergies, asthma, allergic bronchopulmonary aspergillosis and Churg-Strauss syndrome.
3. ***Idiopathic chronic eosinophilic pneumonia*:** As a diagnosis of exclusion, it responds to steroid therapy and manifests with cough, fever, dyspnoea, night sweats and weight

loss. There is consolidation of peripheral lung substance which is infiltrated by lymphocytes and eosinophils (inflammatory cells are present within the alveoli and in the alveolar septae.

Q. Write briefly on smoking-associated interstitial disease.

Ans. Interstitial lung disease associated with smoking includes

1. **Desquamative interstitial pneumonia (DIP):** Airspaces contain aggregates of macrophages which were earlier thought to be desquamated pneumocytes which is why the entity was named DIP. It presents insidiously between 40 and 50 years in smokers with dyspnoea and cough and is steroid responsive.
2. **Respiratory bronchiolitis-associated interstitial lung disease:** A bronchiocentric condition which is typified by peribronciolar chronic inflammation and fibrosis. Pigment containing macrophages are present in the respiratory bronchioles and the patient presents with dyspnoea and cough.

Q. Write briefly on pulmonary alveolar proteinosis (PAP).

Ans. PAP results from a defect in pulmonary macrophage function which results in impaired removal of surfactant from the alveolar and bronchiolar spaces.

Salient Features of PAP

- Rare disease characterized by a defect in GM-CSF or macrophage function
- There is intra-alveolar or intrabronchiolar accumulation of surfactant
- Patient presents with cough and expectoration of gelatinous material
- Histopathology shows consolidation of lung substance due to presence of surfactant containing PAS positive precipitate in the alveolar spaces. There is minimal inflammation with presence of cholesterol clefts.
- PAP may be
  1. **Autoimmune:** Presence of neutralizing antibodies to GM-CSF. Reduced or absent GM-CSF signalling interferes with the terminal differentiation of the alveolar macrophages which in turn impairs their ability to catabolize surfactant
  2. **Secondary:** Occurs secondary to a variety of conditions (hematopoietic disorders, immunodeficiency syndromes, inborn errors of metabolism and acute silicosis
  3. **Hereditary:** Rare neonatal condition caused by GM-CSF receptor gene mutations

Q. Describe the clinicopathological features of occupational lung diseases.

Ans. Occupational lung diseases are a reaction of the lung to inhalation of mineral dusts encountered in the workplace.

- **Factors which determine the extent of damage caused by the inhaled dust:**
  - Size and shape of the particle
  - Their solubility and physiochemical composition
  - The amount of dust retained in the lung
  - The additional effect of other irritants, such as tobacco smoke
  - Host factors, such as efficiency of clearance mechanism and immune status of the host
- **The tissue response to inhaled dust may be of the following three types:**
  - Formation of fibrous nodules
  - Interstitial fibrosis
  - Hypersensitivity reaction

Diseases caused by air pollutants (Table 13.4)

TABLE 13.4. Diseases caused by air pollutants

Agents	Diseases	Exposure
Mineral dusts		
Coal dust	Anthracosis Macules Progressive massive fibrosis (PMF)/ Caplan syndrome	Coal mining
Silica	Silicosis, Caplan syndrome	Sand blasting, stone cutting, foundry workers
Asbestosis	Mesothelioma, carcinoma lung, larynx, colon, pleural plaques	Mining, milling, fabrication
Beryllium	• Acute berylliosis • Beryllium granulomas • May cause bronchogenic carcinoma	Mining, fabrication
Iron oxide	Siderosis	Welding
Barium sulphate	Baritosis	Mining
Tin oxide	Stannosis	Mining, metallurgy, porcelain industry
Organic dusts that cause hypersensitivity pneumonitis		
Moldy hay	Farmer lung	Farming
Bagasse	Bagassosis	Wall board and paper manufacturing
Bird dropping	Bird breeder's lung	Bird handling
Organic dusts that induce asthma		
Cotton, flax, hemp	Byssinosis	Textile manufactures
Chemical fumes and vapours		
NO, SO_2, benzene, NH_3	Bronchitis/ARDS/asthma/ pulmonary oedema/poisoning	Occupational and accidental exposure

1. **Coal workers pneumoconiosis (CWP):** This is the commonest form of occupational disease in coal miners. It may manifest as:
   (a) **Asymptomatic anthracosis**
      (i) Common, benign and asymptomatic accumulation of carbon dust
      (ii) Cigarette smoke and atmospheric pollution increase incidence
      (iii) Alveolar macrophages engulf carbon and accumulate along lymphatics
   (b) **Simple CWP with little or no pulmonary dysfunction**
      (i) Lungs show coal macules (1–2 mm in diameter) or larger coal nodules. These are basically aggregates of dust-laden macrophages with increase in reticulin and collagen.
      (ii) Upper lobes and upper zones of lower lobes are more heavily involved.
      (iii) The macules and nodules are commonly seen adjacent to respiratory bronchioles where dilatation of alveoli leads to **centrilobular emphysema.**
   (c) **Complicated CWP (PMF)**
      (i) Requires many years to develop and is characterized by intensely blackened scars 2–10 cm, usually multiple, bilateral and located more often in the upper parts of the lungs.
      (ii) These masses may break down centrally due to ischaemic necrosis or secondary tuberculous infection.
      (iii) Pleura and regional lymph nodes are blackened and fibrotic. Fibrous lesions are composed of dense collagen and carbon pigment.
      (iv) Wall of respiratory bronchioles and pulmonary vessels are thickened; show scanty inflammatory infiltrate of lymphoid and plasma cells. Alveoli are dilated.
2. **Rheumatoid pneumoconiosis or Caplan syndrome** (rheumatoid arthritis with coal worker's pneumoconiosis, silicosis or asbestosis). Lungs have rounded, firm nodules with central necrosis, cavitation or calcification. Sections from the nodules show fibrinoid necrosis enclosed by palisading mononuclear cells and fibroblasts.

Clinical course of pneumoconiosis

- Simple coal worker's pneumoconiosis usually has a benign course; PMF may lead to cor pulmonale in a few patients.
- Coal dust exposure increases the incidence of chronic bronchitis and emphysema.

***Predisposing factors implicated in the development of PMF*:**

- Older age of the miners
- Amount and duration of exposure to coal dust
- Coexisting tuberculosis
- Coexisting silicosis may further damage the lungs by the following mechanisms:
  - Free radical generation (reactive oxygen species that damage the lung parenchyma)
  - Release of chaemotactic factors, which induce infiltration of inflammatory cells into pulmonary tissue
  - Release of fibrogenic cytokines—IL-1, TNF and PDGF—which cause healing by fibrosis

3. **Silicosis (knife grinder's lung disease)**
   (a) Most prevalent chronic occupational lung disease
   (b) Silica has two forms: crystalline and amorphous; the crystalline forms (quartz, cristobalite and tridymite) are more fibrogenic and toxic than the noncrystalline forms
   (c) Prolonged exposure leads to nodular fibrosing pneumoconiosis

***Pathogenesis* (*Flowchart 13.7*)**

Pathologic changes (knife grinder's lung disease)

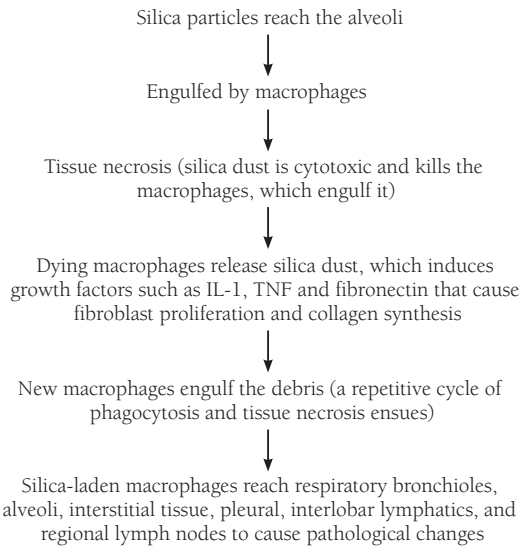
- **Early stages**—tiny, discrete pale to black nodules in the upper zones of the lungs
- **Late stages**—hard, collagen-rich scars, some of which may show central cavitation due to superimposed tuberculosis or ischaemia
- Fibrotic lesions also seen in the **pleura**
- Thin sheets of **calcification** (**'egg shell' calcification)**, noted in lymph nodes
- Progression and PMF ensues
- Microscopy shows concentric layers of hyalinized collagen surrounded by a dense capsule of more condensed collagen. **Polarization** shows birefringent silica particles.

***Clinical features*:**

Patient manifests mainly with dyspnoea.

***Complications*:**

- Pulmonary tuberculosis (silicosis may depress CMI and increases susceptibility to pulmonary tuberculosis)
- Rheumatoid arthritis/Caplan syndrome
- Cor pulmonale
- Lung cancer



FLOWCHART 13.7. Pathogenesis of silicosis.

4. **Asbestos-related disease**
   (a) Prolonged exposure to asbestos dust produces three types of diseases:
      (i) Asbestosis of lungs (parenchymal interstitial fibrosis)
      (ii) Pleural disease (localized fibrous plaques or diffuse fibrosis)
      (iii) Tumours (bronchogenic carcinoma, pleural and peritoneal mesotheliomas, laryngeal carcinoma)
   (b) Asbestos is a family of crystalline hydrated silicates that form fibres which may exist as two distinct geometric forms:
      (i) Serpentine (chrysotile): Curly and flexible fibres (90% of commercial form of asbestos)
      (ii) Amphibole: Straight, stiff and brittle fibres
   (c) Amphiboles are less prevalent but more pathogenic than chrysotile as they are more rigid and less soluble.
   (d) High-risk individuals include miners, millers and fabrication workers

***Pathogenesis* (*Flowchart 13.8*):**

Note: Asbestos reaches the alveoli easily, and has the ability to penetrate epithelial cells leading to diffuse interstitial disease rather than nodular deposits as in silicosis. **Asbestos bodies** are carcinogenic; can act as both initiators and promoters.

***Gross pathology*:**

- Affected lungs are small and firm with marked thickening of the pleura.
- Variable degree of subpleural fibrosis is seen; advanced cases may show cystic changes.
- In contrast to CWP and silicosis, asbestosis begins in the lower lobes and subpleurally.

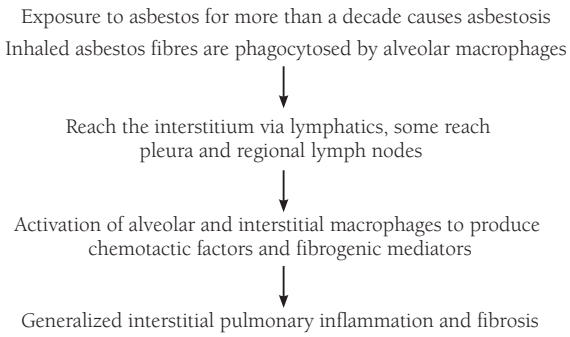
***Microscopy*:**

- Nonspecific interstitial fibrosis with scattered asbestos bodies (asbestos fibres coated with glycoprotein and haemosiderin, which appear as golden brown beaded rods)
- Emphysema is seen in between areas of fibrosis.
- Pleural involvement may result in:
  - Pleural effusion
  - Visceral pleural fibrosis
  - Pleural plaques (most common lesions with asbestos exposure; circumscribed, flat, 1 cm, firm-to-hard bilateral nodules)

Clinical features

- Slow insidious illness, which may be asymptomatic for years or may be present with dyspnoea and dry or productive cough.
- Pulmonary hypertension and cor pulmonale are observed in advanced cases.

5. **Berylliosis**
   (a) Due to heavy exposure to dust/fumes of metallic beryllium or its salts
   (b) Used in nuclear, electronic and aerospace industries
      (i) **Acute berylliosis**
         - Seen after 2–4 weeks of exposure



FLOWCHART 13.8. Pathogenesis of asbestosis.

- Manifests with dyspnoea, hypercapnia and chest pain due to filling up of alveoli with protein-rich fluid and formation of hyaline membrane
- Complete recovery may be seen

(ii) **Chronic berylliosis**

- Seen after 20 years or more of exposure
- Cell-mediated hypersensitivity reaction produces noncaseating granulomas

Q. Define cor pulmonale. Enumerate the types of cor pulmonale.

Ans. Cor pulmonale is dilatation (with or without hypertrophy) of right ventricle due to a primary respiratory disorder.

- Hypertrophy is a feature of chronic cor pulmonale; whereas, dilatation dominates in acute cor pulmonale.
- Pulmonary hypertension is the common link between heart and lung dysfunction.

Q. What is acute cor pulmonale? Discuss the clinical manifestations of acute cor pulmonale.

Ans. Acute cor pulmonale usually follows acute massive pulmonary embolism, which is sufficient enough to obstruct more than 60% of pulmonary circulation. It leads to acute pulmonary hypertension, acute right ventricular dilatation and failure.

Q. Discuss the aetiopathogenesis and clinicopathological features of chronic cor pulmonale.

Ans. Chronic cor pulmonale is defined as a combination of hypertrophy and dilatation of the right ventricle secondary to pulmonary hypertension, which results from diseases of lung, pulmonary circulation or thorax.

Aetiology

- Chronic obstructive pulmonary disease (COPD—including chronic bronchitis and emphysema) are responsible for more than 50% cases of chronic cor pulmonale.
- Early onset of cor pulmonale is seen in patients with chronic bronchitis (blue bloaters).
- The onset of cor pulmonale is late in patients with emphysema (pink puffers).
- Increased pulmonary vascular resistance and pulmonary hypertension are the central mechanisms in all cases of chronic cor pulmonale.

Clinical Features

- Dyspnoea, due to pulmonary hypertension, not relieved by sitting up.
- Dry cough
- Chest pain due to dilatation of the root of pulmonary artery
- Exercise-induced peripheral cyanosis
- **Signs of overt right heart failure** including peripheral oedema, raised jugular venous pressure, tender hepatomegaly, cardiac enlargement, right ventricular third heart sound and a gallop rhythm.

Q. Classify lung tumours. Briefly describe their aetiopathogenesis and morphology.

Ans. Tumours of lung include

- Malignant epithelial tumours or carcinomas (which constitute 90–95% of lung tumours)
- Bronchial carcinoids (which constitute 5% of all lung tumours)
- Mesenchymal and miscellaneous tumours (which constitute 2–5% of all lung tumours)

Classification of malignant epithelial tumours

1. **Histological classification**
   (a) Squamous cell carcinoma (papillary, clear cell, small cell and basaloid)
   (b) Adenocarcinoma (most common)
      (i) Minimally invasive adenocarcinoma (nonmucinous and mucinous)
      (ii) Lepidic, acinar, papillary, solid (according to pattern of arrangement of tumour cells)
      (iii) Mucinous adenocarcinoma
   (c) Small cell carcinoma
   Combined small cell carcinoma
   (d) Large cell carcinoma: Large cell–neuroendocrine carcinoma
   (e) Adenosquamous carcinoma
   (f) Carcinomas with pleomorphic, sarcomatoid or sarcomatous elements
   (g) Carcinoid tumour: Typical and atypical
   (h) Carcinoma of salivary gland origin
   (i) Unclassified carcinomas
2. **Therapy-based classification**
   (a) Small cell carcinoma (aggressive; show a high initial response to chemotherapy)
   (b) Non–small cell carcinoma (have a better prognosis than small cell carcinomas)

Epidemiology

- Adenocarcinoma is the most common lung carcinoma in females; incidence of adenocarcinoma has increased over the past few years (increase in incidence is thought to be due to increase in the number of female smokers).
- Strongest relationship with smoking is seen in squamous cell and small cell carcinoma.
- Bronchogenic carcinoma is the most frequently fatal malignancy with a peak incidence between 40 and 70 years.

Aetiology and Pathogenesis

1. **Role of tobacco smoking**:
   (a) Invariable statistical association with:
      - Amount of daily smoking and tendency to inhale
      - Duration of habit (heavy smokers smoking more than 40 cigarettes/day for many years have a 20-fold increased risk)

   Eight percent incidence of lung carcinoma occurs in smokers. Cigar and pipe smoking associated with less risk.

   Note: Other smoking-associated cancers: cancer of lip, tongue, floor of mouth, pharynx, larynx, oesophagus, urinary bladder, pancreas and kidney

   (b) Documentation of precursor histological changes (hyperplasia and dysplasia) in lining epithelium of respiratory tract in smokers.
   (c) Experimental work has revealed presence of more than 1200 harmful substances found in tobacco smoke, eg,
      (i) Initiators like polycyclic aromatic hydrocarbons (benzopyrene)
      (ii) Promoters such as phenol derivatives
      (iii) Radioactive elements—polonium-210, carbon-14 and potassium-40
      (iv) Arsenic, nickel and molds

   Not all people exposed to tobacco smoke, however, develop lung cancer. It is therefore hypothesized that the mutagenic effect of tobacco smoke is dependant on genetic variants (the procarcinogens present in tobacco smoke are converted to carcinogens by P-450 monooxygenase enzyme. Specific P-450 polymorphisms have an enhanced ability to activate them, making smokers with these genetic variants more susceptible to lung cancer).
2. **Industrial hazards associated with lung carcinoma:**
   (a) Radiation
   (b) Uranium (miners)

(c) Asbestosis (particularly, when coupled with smoking)
(d) Nickel, chromates, coal, mustard and arsenic

3. **Indoor air pollutants** (eg, radon) have been implicated.
4. **Role of molecular genetics:**
   (a) **Loss of tumour suppressor genes like P53, RB1** and **inactivation of CDK inhibitor P_{16}** are seen equally in adeno and squamous cell carcinoma. P53 and RB1 mutations are common in small cell carcinoma as well. Small cell carcinomas also commonly demonstrate amplification of genes of MYC family.
   (b) Gain of function mutations involving the **growth factor receptor signalling pathways (genes encoding receptor tyrosine kinases, eg, EGFR, ALK, ROS, MET and RET)**, amplifications in **epidermal growth factor receptor (EGFR) gene** and mutations in **KRAS** are typically seen in patients with **adenocarcinoma.**
   (c) Allelic losses on short arm of chromosomes 3, 9 and 17 may precede invasion in squamous cell carcinoma.
5. **Scarring**
   (a) Scars are most often encountered in the vicinity of adenocarcinomas.
   (b) In most cases, scar is a desmoplastic response to tumour; occasionally, scar may precede carcinoma (old infarcts, foreign bodies, wounds and granulomatous inflammation).

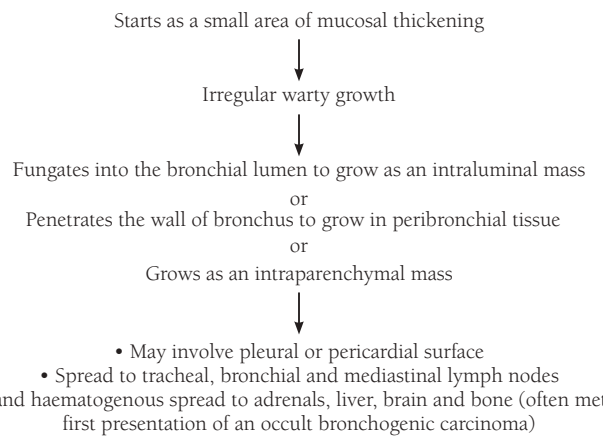
Four types of Precursor Lesions are recognized:

1) Squamous dysplasia and carcinoma in situ
2) Atypical adenomatous hyperplasia (small < 5 mm lesions, solitary or multiple, composed of dysplastic pneumocytes lining fibrosed alveolar walls)
3) Adenocarcinoma in situ (formerly called bronchioloalveolar carcinoma it is a lesion smaller than 3 cm. It is constituted by dysplastic cells which grow along alveolar septa.)
4) Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia.

Morphology

- Most common location is the hilar region.
- Most lesions arise from 1st, 2nd and 3rd order bronchi; few arise from peripheral alveolar septal cells and terminal bronchioles.
- Peripheral tumours are usually adenocarcinomas.

Evolution (Flowchart 13.9)



FLOWCHART 13.9. Evolution of bronchogenic carcinoma.

1. **Squamous cell carcinoma**
   (a) More common in males
   (b) Strong association with smoking
   (c) Usually central in location; recent increase in the incidence of peripheral lesions

(d) Well-differentiated lesions show minimal atypia, intercellular bridges as well as abundant keratin (keratinization is seen as numerous keratin pearls as well as individual cell keratinization. Squamous cells with intracellular keratin demonstrate abundant dense eosinophilic cytoplasm).
(e) Moderately differentiated lesions show moderate atypia, individual cell keratinization, occasional keratin pearl, if any and fewer intercellular bridges (Fig. 13.6).
(f) Poorly differentiated lesions are focally keratinized (do not demonstrate keratin easily) and show severe atypia. These lesions are difficult to recognize as squamous in origin.
(g) Squamous metaplasia, dysplasia and squamous cell carcinoma in situ may be seen in the adjacent tissue.

2. **Adenocarcinoma.** It is of two types:
(a) Most common carcinoma in females and nonsmokers
(b) Peripheral/smaller/slow growing
(c) Well-differentiated tumours show well-formed glands with occasional papillary differentiation and easily demonstrable mucin.
(d) Poorly differentiated lesions show minimal gland formation with solid sheets of poorly differentiated cells, which require special stains/immunohistochemistry to demonstrate foci of mucin-producing cells.
(e) In the lepidic pattern, tumour cells crawl along the alveolar septae which tend to maintain their architecture.
(f) Tumours less than 3 cm with an invasive component less than 5 mm, associated with a peripheral lepidic pattern and scarring is labelled microinvasive adenocarcinoma. Mucinous adenocarcinoma spread easily forming satellite nodules.

3. **Small cell carcinoma**
(a) Highly malignant tumour; metastasizes widely and has a strong association with cigarette smoking
(b) May be hilar or central
(c) Epithelial cells are small, round to oval with scanty cytoplasm appear lymphocyte like (but twice the size of a small lymphocyte; Fig. 13.7) and are called oat cells. Occasionally, they may be spindle shaped or polygonal.
(d) Necrosis and mitotic activity are common. Basophilic staining of vessel walls is commonly seen due to smudging by DNA from necrotic cells (Azzopardi effect).
(e) Nuclear moulding is prominent and results from close apposition of tumour cells that have scanty cytoplasm.

Electron microscopy
- Tumour cells demonstrate dense core neurosecretory granules
- Thought to be derived from neuroendocrine or Kulchitsky cells
- Positive for chromogranin, synaptophysin, CD 57, NSE, PTAH and polypeptide hormones

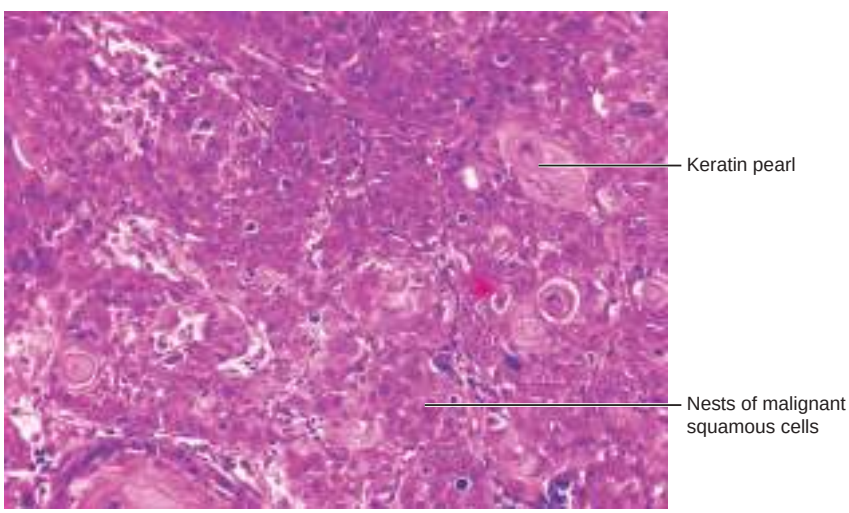


FIGURE 13.6. Moderately differentiated squamous cell carcinoma showing moderate atypia, individual cell keratinization and occasional keratin pearl, formation (H&E; 200×).

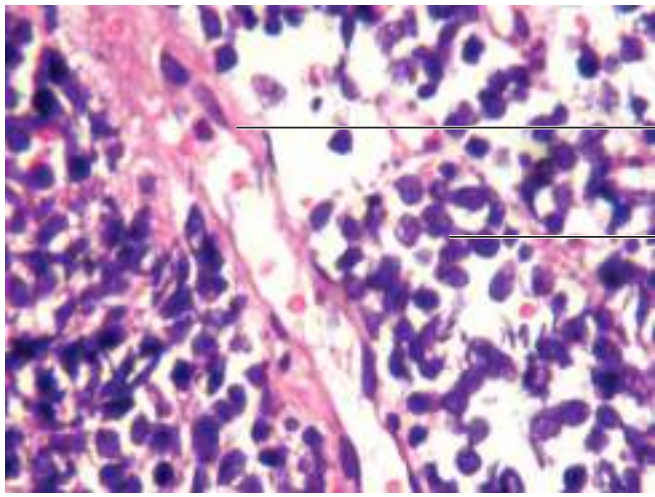


FIGURE 13.7. Section from small cell carcinoma of lung showing round-to-oval tumour cells with scanty cytoplasm and hyperchromatic crowded nuclei (H&E; 400×).

4. **Large cell carcinoma**
   (a) Large anaplastic polygonal cells with vesicular nuclei (thought to be undifferentiated squamous and adenocarcinomas which can no longer be recognized on light microscopy)
   (b) ***Variants*:** Giant cell, clear cell, spindle cell and large cell neuroendocrine carcinoma

About 10% lung carcinomas have a combined morphology with two or more histological types.

Consequences of Bronchogenic Carcinoma

- Emphysema (due to partial obstruction of airways by the tumour)
- Atelectasis (due to total obstruction of airways by the tumour)
- Suppurative/ulcerative bronchitis or bronchiectasis or pulmonary abscess (due to impaired drainage of airways)
- Venous congestion or **dusky head** (due to compression or invasion of superior vena cava)
- Haemoptysis (due to haemorrhage from tumour in the airway)
- Pleural effusion, pericarditis or tamponade (due to extension of tumour to pleural/pericardial sac)
- Apical tumours invade into brachial or cervical sympathetic plexus causing pain in the region of ulnar nerve or **Horner syndrome (ipsilateral enophthalmos, ptosis, miosis and anhidrosis)**. May be accompanied by destruction of first and second ribs and sometimes thoracic vertebrae **(Pancoast syndrome).**
- Hoarseness (due to recurrent laryngeal nerve invasion), dysphagia (due to oesophageal invasion) and diaphragmatic paralysis (due to phrenic nerve invasion)

Diagnosis of Lung Carcinoma

Common Symptoms:

Cough, weight loss, chest pain and dyspnoea

Investigations

- X-ray chest
- Ultrasound or C.T. guided FNAC/biopsy

Paraneoplastic Syndromes Associated with Bronchogenic Carcinoma

Various hormones or hormone-like substances are secreted by bronchogenic carcinoma, eg,

- ADH leading to hypernatraemia
- ACTH leading to Cushing syndrome

- PTH causing hyperkalaemia
- Calcitonin leading to hypocalcaemia
- Gonadotrophins causing gynaecomastia
- Serotonin-inducing carcinoid syndrome

Other Systemic Manifestations of Lung Carcinoma

- **Lambert–Eaton myasthenic syndrome** (a rare autoimmune disorder associated with small cell carcinoma that is characterized by muscle weakness of the limbs resulting from an autoimmune reaction, where antibodies are formed against voltage-gated calcium channels in the neuromuscular junction)
- **Sensory type of neuropathy**
- **Acanthosis nigricans** (brown to black, velvety hyperpigmentation of the skin usually found in body folds)
- **Leukemoid reactions**
- **Hypertrophic pulmonary osteoarthropathy** (clinical triad of digital clubbing, arthralgias, and ossifying periostitis)

Prognosis

- Five-year survival rate:
  - **Squamous and adenocarcinoma** → 10%
  - **Small cell carcinoma** → few weeks in untreated patients
- Surgery ineffective in **bronchioloalveolar carcinoma** (responsive to chemotherapy and radiotherapy)
- Solitary lesions can be removed surgically and have a better survival than multiple/pneumonic lesions.

Neuroendocrine Tumours of Lung

1. Benign tumours
2. Carcinoids
3. Small cell carcinoma

Bronchial Carcinoids

1. Constitute 1–5% of all lung tumours
2. Patients affected are generally young
3. No relationship with smoking/environmental factors
4. They present as finger-like or spherical polypoid masses, projecting into the lumen, and covered by mucosa. They are rarely more than 3–4 cm
5. Most carcinoids remain confined to main stem bronchus; some intraluminal masses show infiltration into the peribronchial tissue (collar button lesion)
6. Typical carcinoids have less than 2 mitoses per 10 high power fields and absence of necrosis while atypical carcinoids have between 2 and 10 mitoses per high power field and foci of necrosis.

Metastatic Tumours of Lung

- The lung is the most common site for metastases for both carcinomas and sarcomas. Local spread may occur from oesophagus and mediastinum.
- Common sources of epithelial metastases include GIT, breast, thyroid, kidney, pancreas and liver.
- Other tumours which frequently metastasize to lungs include osteogenic sarcoma, melanoma, leukaemia-lymphomas, neuroblastoma and Wilms tumour.
- Metastasis usually presents as multiple nodules throughout the lung substance, more towards the periphery; when large, they are labelled canon ball metastasis. Rarely, it may present as a solitary nodule or pneumonic consolidation.

14

The Oral Cavity and Gastrointestinal Tract

ORAL CAVITY

- The process of digestion starts in the oral cavity, which is the beginning of the gastrointestinal tract (GIT). It has many supporting structures, like the lips, teeth and tongue. Oral cavity has two main parts: the outer portion or, the vestibule, and an inner mouth cavity. The vestibule (space between the cheeks and the lips) is smaller than the oral cavity proper. The stratified squamous nonkeratinized epithelium lining the oral mucosa changes to stratified squamous keratinized epithelium in the lips.
- The boundaries of the oral cavity include the alveolar arches and teeth (lateral and front), the pharynx (behind) and the palate (superiorly). The palate consists of two regions: the anterior two-third or bony part, called the hard palate and the posterior one-third or fibromuscular part, known as the soft palate. The palate is also lined by stratified squamous nonkeratinized epithelium.
- The bones that are part of the oral cavity are the maxilla, mandible and the hard palate. The hard palate is formed by the palatine process of the maxilla and the maxillary process of the palatine bones.

Q. Write briefly on tumours and tumour-like lesions of oral cavity.

Ans. Benign Tumours and Tumour-Like Lesions

- Common '**tumour-like lesions**' of the oral cavity include pyogenic granulomas, fibroepithelial polyps, fibrous epulis, denture hyperplasia and mucoceles.
- **Benign tumours** in the oral cavity may arise from the following:
  1. Squamous epithelium
  2. Mesenchymal tissue
  3. Minor salivary glands
- The most common benign epithelial neoplasm is **squamous papilloma**. It is a small, cauliflower-like, sessile or pedunculated lesion having a central fibrovascular core covered by hyperplastic (acanthotic), stratified squamous epithelium. Most of these are viral in origin and show 'koilocytosis'. Koilocytes are defined as cells showing a hyperchromatic nucleus with irregular nuclear membrane surrounded by a clear zone. Other common epithelium-derived neoplasms in this location are **tumours of the minor salivary glands.**
- Benign mesenchymal tumours include **haemangioma, lymphangioma, fibroma, lipoma, neural tumours**, etc.
- **Granular cell tumour** (earlier called granular cell myoblastoma) is a mesenchymal tumour of the skin and mucosal surfaces. In the oral cavity, it is most commonly located in the dorsum of the tongue. The tumour comprises large polygonal cells, which have abundant granular cytoplasm containing cytoplasmic inclusions. The epithelium overlying the tumour may show pseudoepitheliomatous hyperplasia.

Precancerous Lesions

The most relevant precancerous lesions are

Leukoplakia

The term leukoplakia is defined by the World Health Organization (WHO) as, 'a white patch/plaque that cannot be scraped off and cannot be characterized clinically or pathologically'. Approximately, 5–25% of these lesions are premalignant. Thus, until proved otherwise by histological evaluation, all leukoplakic patches must be considered precancerous.

Differential diagnosis of white lesions in the oral cavity:

- Reactive epithelial hyperplasias (hyperorthokeratosis, parakeratosis and acanthosis)
- Leukoplakia of infective origin (candida, syphilis and hairy leukoplakia associated with Epstein–Barr virus)
- Lichen planus
- Oral submucous fibrosis
- Lupus erythematosus
- Congenital lesions (eg, white sponge nevus, dyskeratosis congenita and pachyonychia congenita)
- Invasive carcinoma

Morphology

- Leukoplakic patches are mostly seen on the cheek (buccal) mucosa, angles and floor of the mouth, tongue, palate and gingiva.
- They may be solitary or multiple, and are of variable size and shape.
- Microscopic examination shows varied histopathology ranging from hyperkeratosis and/or acanthosis without atypia to lesions with marked dysplasia, sometimes merging into carcinoma in situ or invasive carcinoma.

Erythroplakia

- Erythroplakia indicates a red patch that is difficult to categorize clinically as any established disease entity. It usually presents as, a well-defined, velvety, granular or nodular lesion in the soft palate, floor of mouth, ventral surface of the tongue and retromolar area.
- Erythroplakia almost always presents with superficial erosions; epidermal thickening is unusual.
- Histologically, these lesions are more aggressive compared with leukoplakic lesions and show changes varying from dysplasia, carcinoma in situ, to frankly invasive carcinoma. The red colour of the lesion is due to marked subepithelial inflammation and dilatation of submucosal vessels.

Squamous Cell Carcinoma

Squamous cell carcinomas (SCCs) comprise almost 95% of cancers of the head and neck (HNSCCs) and are most commonly located in the oral cavity.

Pathogenesis

- The pathogenesis of SCC is multifactorial; smoking and alcohol in excess, inherited genomic instability, persistent irritation and human papilloma virus (HPV types 16, 18 and 33) infection are all implicated.
- Actinic radiation (sunlight), pipe smoking, chewing of betel and arecanuts are the known predisposing factors.
- SCC evolves through a multistep process in which activation of oncogenes and inactivation of tumour suppressor genes are simultaneously ongoing.

Morphology

- Oral SCC may present as an ulcertive, verrucous or nodular plaque-like lesion, often seen developing in a pre-existing leukoplakic or erythroplakic lesion.
- It usually begins as a dysplastic lesion, which progresses to carcinoma in situ and then invasive carcinoma.

- SCCs range from well-differentiated keratinizing neoplasms to poorly differentiated, anaplastic, sometimes sarcomatoid tumours (spindle cell variant of SCC).
- Local metastasis preferentially occurs in the cervical lymph nodes, while distant metastasis is most commonly noted in mediastinal lymph nodes, lungs, liver and bones.

SALIVARY GLANDS

- There are three major salivary glands—parotid, submandibular and sublingual—as well as innumerable minor salivary glands distributed throughout the mucosa of the oral cavity. All these glands are subject to inflammation or to development of neoplasms.
- Salivary glands are compound exocrine glands with ductal and acinar portions. The acinar portion may be serous, mucinous or mixed; and, all acini are lined by luminal cells, which are enclosed by myoepithelial cells.
- Serous acini have dense, basophilic, Periodic acid–Schiff (PAS)-positive intracytoplasmic secretory granules containing amylase and a small central lumen.
- Mucous acini are larger than serous acini; have cells with abundant cytoplasm containing mucin, well-rounded basal nuclei and are arranged around empty lumina; produce acidic mucins (positive for alcian blue and mucicarmine) and neutral mucins (positive for PAS).
- Myoepithelial cells surround acini and intercalated ducts, and mediate contraction.
- Ducts are intercalated, striated or interlobular, all with outer basal cells and inner luminal cells. Intercalated ducts have reserve cells that regenerate acinar tissue and terminal duct system.

Q. Write briefly on salivary gland tumours.

Ans. Salivary gland tumours (SGTs) are relatively uncommon; they constitute only about 2% of all head and neck neoplasms. Nearly 80% of these tumours occur in the parotid glands, 15% in the submandibular glands and the remaining 5% in the sublingual and minor salivary glands. Benign neoplasms make up about 80% of parotid tumours, 50% of submandibular tumours and less than 40% of sublingual and minor salivary gland tumours.

Classification

Benign epithelial tumours:

- Pleomorphic adenoma
- Myoepithelioma
- Basal cell adenoma
- Warthin tumour
- Oncocytoma
- Canalicular adenoma
- Sebaceous adenoma
- Lymph adenoma
- Ductal papilloma
- Cystadenoma

Malignant epithelial tumours:

- Acinic cell carcinoma
- Mucoepidermoid carcinoma
- Adenoid cystic carcinoma
- Polymorphous low-grade adenocarcinoma
- Epithelial–myoepithelial carcinoma
- Clear-cell carcinoma; not otherwise specified
- Basal-cell adenocarcinoma
- Malignant sebaceous tumours
- Cystadenocarcinoma
- Low-grade cribriform cystadenocarcinoma

- Mucinous adenocarcinoma
- Oncocytic carcinoma
- Salivary duct carcinoma
- Adenocarcinoma; not otherwise specified
- Myoepithelial carcinoma
- Carcinoma expleomorphic adenoma
- Carcinosarcoma
- Metastasizing pleomorphic adenoma
- Squamous cell carcinoma
- Small-cell carcinoma
- Large-cell carcinoma
- Lymphoepithelial carcinoma

Mixed Parotid Tumour (Pleomorphic Adenoma)

- Accounts for more than 90% of the benign tumours of salivary glands
- Presents as painless swelling at angle of the jaw
- Most common location is superficial lobe of parotid followed by the submandibular gland. It is rare in minor salivary glands.
- Most often diagnosed in the fourth to sixth decades of life, it is uncommon in children. Women are more frequently affected.
- Thought to originate from epithelial/myoepithelial/ductal reserve cells.

Gross Morphology

- Small, well-demarcated, round and multilobulated lesion.
- Appears well encapsulated, but on close inspection shows finger-like extensions across the tumour capsule at multiple sites.
- They are typically solid, but cut surface has a variegated appearance; may be grey-white, myxoid, with blue, translucent pseudochondroid areas.

Microscopic Features (Fig. 14.1A and B)

- Pleomorphic adenomas show both epithelial and mesenchymal differentiation; were also called mixed tumours because they were thought to arise from more than one germ cell layer. They can undergo secondary malignant change.
- Epithelial component (ductal and myoepithelial cells) forms ducts, acini, tubules, strands or sheets. Ductal cells are cuboidal; myoepithelial cells are flattened or spindled.
- Background stroma may be mucoid, myxoid, pseudochondroid or hyaline

Warthin Tumour

- Also called papillary cystadenoma lymphomatosum, it is a benign tumour seen exclusively in the parotid gland.
- Usually affects males in the fifth to seventh decades of life.
- May be multicentric
- Histogenesis is disputed, but it is thought to arise from heterotopic salivary tissue trapped in a regional lymph node during embryogenesis.

Gross Morphology

- Arises in superficial parotid gland; is small, round-to-oval, lobulated and encapsulated.
- Mucin-containing narrow cysts or cleft (slit)-like spaces showing papillary projections may be seen on the cut surface.

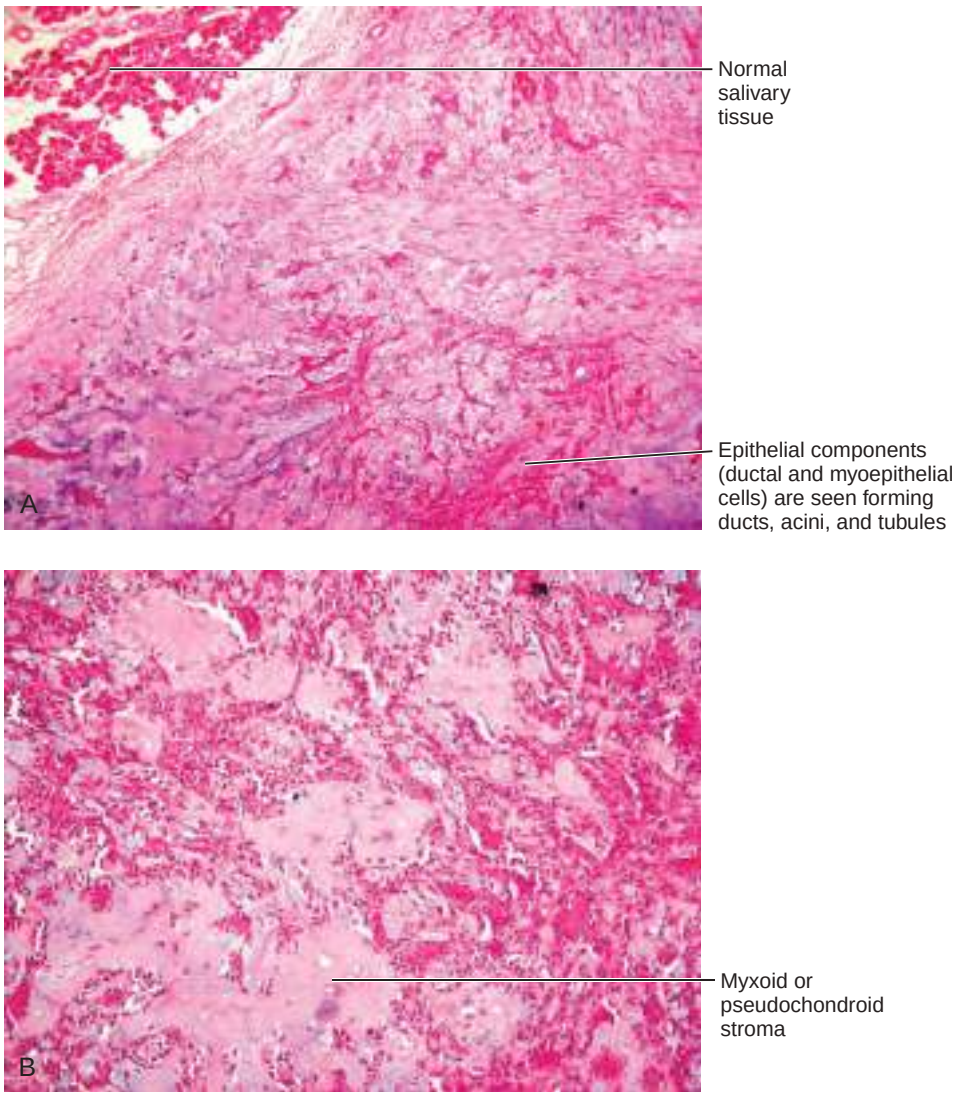


FIGURE 14.1. (A) Section from a pleomorphic adenoma showing both epithelial and mesenchymal elements. Epithelial components (ductal and myoepithelial cells) are seen forming ducts, acini, tubules, strands or sheets. Ductal cells are cuboidal; myoepithelial cells are flattened or spindled (H&E; 200×). (B) Pleomorphic adenoma showing epithelial and myoepithelial cells lying against a myxoid or pseudochondroid stroma (H&E; 100×).

Microscopic Features (Fig. 14.2)

- Warthin tumour is a biphasic tumour showing epithelial and myoepithelial components, along with a lymphoid stroma.
- Epithelial components include
  - Glandular or cystic structures lined by double-layer epithelium
  - The inner cell layer is of columnar cells with abundant, finely granular and cytoplasm (oncocytic cells).
  - The outer cell layer is cuboidal to polygonal.
  - Secretory cells are dispersed in inner layer of columnar cells.
  - Spindle-shaped or flattened cells constitute myoepithelial component
- Lymphoid stroma is present under the epithelium in the form of lymphoid follicles, often with germinal centres.

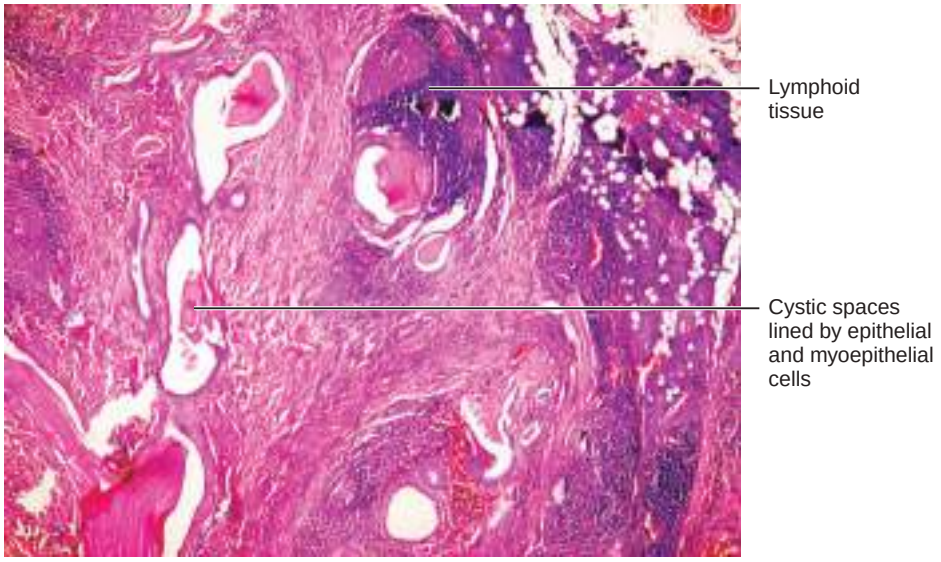


FIGURE 14.2. Section from Warthin tumour showing epithelial and myoepithelial cells in a lymphoid stroma (H&E; 40×).

Monomorphic Adenoma

- This tumour is similar to pleomorphic adenoma, except it does not contain a mesenchymal stromal component.
- It is more common in minor salivary glands (eg, upper lip), is bilateral in about 10% cases, and has a very rare malignant potential.
- Types include
  - Basal-cell adenoma (most common)
  - Canicular adenoma
  - Myoepithelioma adenoma
  - Clear-cell adenoma
  - Membranous adenoma
  - Glycogen-rich adenoma

Basal cell adenomas are well-encapsulated, smooth tumours on gross inspection, and are divided into four subtypes based on their microscopic appearance—solid, trabecular, tubular and membranous. The constituent tumour cells are monomorphic basaloid epithelial cells that show peripheral nuclear palisading, and have hyperchromatic, round nuclei and indistinct cytoplasm.

Mucoepidermoid Carcinoma (MEC)

- MEC is the most common malignant tumour of the parotid gland and the second most common malignancy (adenoid cystic carcinoma is more common) of the submandibular and minor salivary glands.
- On gross inspection, some MECs appear well circumscribed and may be partially encapsulated. Others are poorly defined and infiltrative.
- The cut surface of the tumour may contain solid areas, cystic areas or both. The cystic spaces contain viscous or mucoid material.
- Microscopically (Fig. 14.3); these tumours are characterized by presence of two populations of cells—the mucous cells and the epidermoid/squamous cells, the proportion of which helps to define grade of the tumour. MEC may be low grade (well differentiated) or high grade (poorly differentiated).
- Low-grade MEC has prominent cystic structures and proportionally more mucous cells, which may form gland-like structures and fewer epidermoid cells.

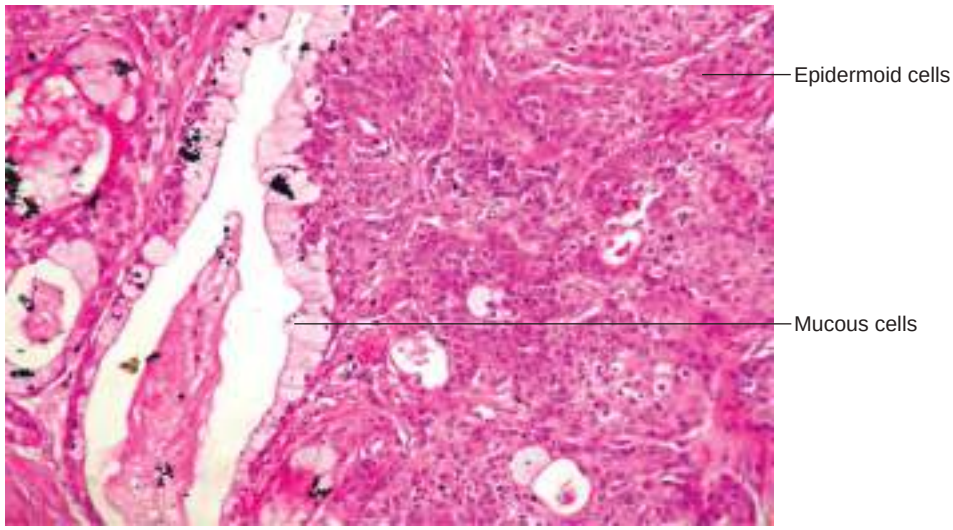


FIGURE 14.3. Low-grade mucoepidermoid carcinoma displaying both an epidermoid component and cystic spaces lined by mucous cells (H&E; 200×).

- Intermediate-grade tumours display fewer cysts and a substantial solid component. Although mucous cells are still present, there is an increasing proportion of epidermoid cells and occasional keratin pearl formation.
- The high-grade carcinomas are solid tumours comprised mainly of epidermoid cells that show prominent cellular atypia and mitoses. These tumours can be mistaken for an SCC. A positive immunohistochemical staining for mucin indicates a high-grade mucoepidermoid carcinoma, rather than an SCC.
- Therapy for MEC depends on the stage, grade and location of the tumour. Stages I and II disease can often be treated by surgical excision alone (parotidectomy with facial nerve preservation, submandibular gland excision or wide local excision of an involved minor salivary gland). Stages III and IV disease require radical excision and may warrant additional intervention such as a neck dissection or postoperative radiation therapy.

Adenoid Cystic Carcinoma

- It peaks in fifth decade of life, and presents as a gradually enlarging salivary mass, which may be accompanied by pain and paraesthesias.
- On gross inspection the tumour appears well defined but unencapsulated. In late stages, the tumour can be seen infiltrating the surrounding normal tissue. Contrary to the name, these tumours are solid in consistency and rarely display cystic spaces on the cut surface.
- The tumour is composed of epithelial and myoepithelial cells variably arranged in tubular, cribriform and solid patterns. The cribriform pattern is the most common and easily recognizable. It is often referred to as 'Swiss-cheese' pattern. Tumour cells are arranged in nests around cylindrical spaces that may contain a mucinous or hyalinized material. Cells that are arranged in layers and form ductal structures characterize tubular pattern. The solid pattern contains sheets of tumour cells with no intervening spaces (Fig. 14.4).
- Current treatment recommendations for adenoid cystic carcinoma include complete surgical resection and postoperative radiation therapy. Because of the propensity for this tumour to demonstrate perineural invasion, sacrifice of the facial nerve may be necessary for tumour eradication.

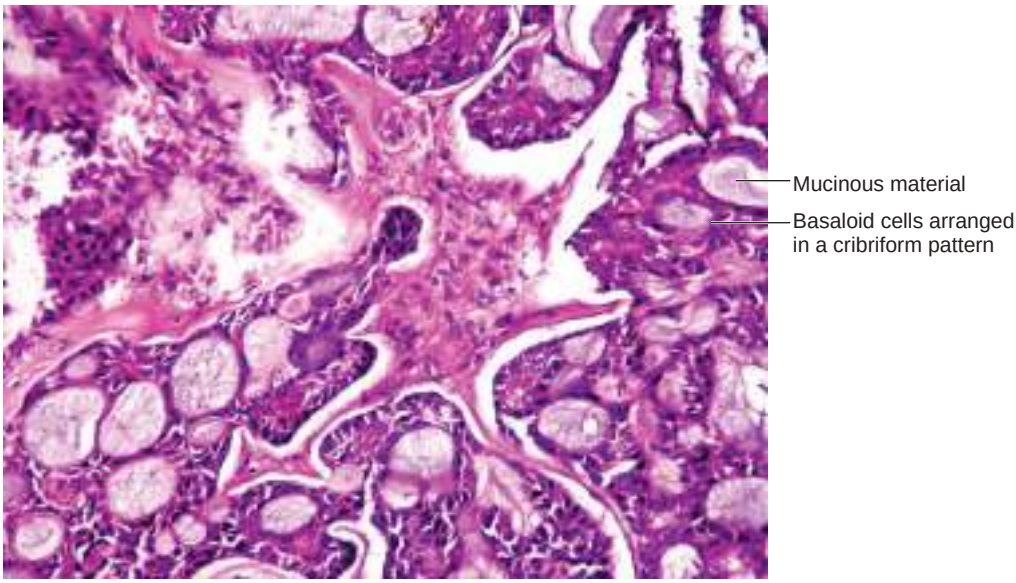


FIGURE 14.4. Adenoid cystic carcinoma showing tumour cells arranged in a cribriform pattern around cylindrical spaces that contain mucinous material (H&E; 200×).

Acinic Cell Carcinoma

- Acinic cell carcinoma is a rare tumour that accounts for about 1% of all salivary neoplasms.
- It typically presents in the fifth decade of life, and is more common in women. Bilateral parotid disease occurs in approximately 3% of cases.
- Most common presentation is that of an asymptomatic enlarging mass.
- Gross appearance demonstrates a mass that is well circumscribed but lacks a true capsule.
- Acinic cell carcinoma is a malignant neoplasm demonstrating serous acinar cell differentiation. Acinar cells are large, polygonal with lightly basophilic, granular cytoplasm and round, eccentric nucleus. The cytoplasmic zymogen secretory granules are PAS-positive, resistant to diastase digestion and nonreactive or only weakly reactive to mucicarmine stain.
- This tumour is generally regarded as a low-grade malignancy. Treatment is surgical excision.

Adenocarcinoma

- Adenocarcinomas of the salivary glands are rare but aggressive tumours.
- They are most common in the parotid followed by the minor salivary glands.
- Microscopically there is formation of glandular structures, and based on the degree of differentiation they are described as grades I, II or III tumours. Grade I lesions have well-formed ductal structures, while Grade III lesions have a more solid growth pattern with few glandular characteristics.
- Treatment for adenocarcinoma is aggressive. Complete local excision with facial nerve sacrifice, partial resection of the maxilla or mandible, postoperative radiation therapy and neck dissection is warranted in most cases.

Malignant Mixed Tumours

- Carcinoma expleomorphic adenoma is most common. It occurs when a carcinoma develops from the epithelial component of a pre-existing pleomorphic adenoma. The other two tumours in this category, carcinosarcoma and metastasizing mixed tumour, are much less common.
- In a carcinosarcoma, the metastatic lesions contain both the stromal and epithelial elements. This is different from the carcinoma expleomorphic adenoma in which only the epithelial elements are present in metastasis. The metastasizing mixed

tumour refers to an otherwise benign pleomorphic adenoma that develops metastatic deposits of tumour.
- Microscopically, carcinoma expleomorphic adenoma most often is an undifferentiated carcinoma (30%) or adenocarcinoma (25%). This tumour tends to be more aggressive than other salivary malignancies.

Polymorphous Low-Grade Adenocarcinoma

- Polymorphous low-grade adenocarcinoma (PLGA) is the second most common malignancy in the minor salivary glands and occurs most frequently in the palate, lip and buccal mucosa.
- This tumour typically presents in the seventh decade of life and is more common in women.
- True to its name, any growth pattern (solid, tubular, trabecular, glandular, cribriform and cystic) can be seen within the same lesion or in different lesions.
- PLGA displays a tendency for perineural and perivascular invasion; however, it typically follows an indolent course. Treatment is complete local excision.

OESOPHAGUS

Adult oesophagus is 24–30 cm in length from cricoid to oesophagogastric junction and 38–40 cm from dental incisors. For the purpose of classification, staging and reporting of oesophageal carcinoma, oesophagus is divided into four segments, namely:

(a) **Cervical:** Cricoid to thoracic inlet
(b) **Upper thoracic segment:** Thoracic inlet to tracheal bifurcation
(c) **Mid-thoracic segment:** Tracheal bifurcation to 8th cervical vertebra
(d) **Lower thoracic segment:** 8th cervical vertebra to the stomach

Histology

- Oesophageal mucosa is lined by nonkeratinized stratified squamous epithelium.
- Lamina propria is composed of connective tissue.
- Muscularis mucosae is thicker than in the other parts of GIT.
- Mucous glands are present in the uppermost and lowermost regions; glands in the lowermost region resemble cardiac glands of the stomach.
- Submucosa is composed of branched tubular mucous glands throughout.
- Muscularis externa is composed variably of:
  - Striated muscle (forms pharyngoesophageal sphincter) in the upper one-third
  - Has both smooth and striated muscles in the middle one-third
  - Smooth muscle in the lower one-third (forms lower oesophageal sphincter (LES) lower one-third)
  - Muscularis externa has two layers—outer longitudinal and inner circular
- Adventitia has connective tissue blending with the surrounding tissue.

Q. Write briefly on achalasia cardia.

Ans. Achalasia cardia is caused by a failure of relaxation of LES and has the following features:

- Complete absence of peristalsis and elevation of resting LES pressure or low amplitude nonperistaltic contractions
- Increased intraoesophageal pressure
- Functional obstruction of oesophagus with a dilated fluid and food-filled proximal portion

Age

20–40 years

Symptoms

Progressive dysphagia for solids and liquids, regurgitation, chest pain and weight loss

Aetiology

- **Primary achalasia:** Aetiology unknown (may be neuronal rather than a myopathic disorder). Number of ganglion cells have been found to be decreased in Auerbach's plexus (ganglion cell degeneration).
- **Secondary forms:** Typically seen in Chagas disease (Trypanosoma cruzi infection), polio, diabetic autonomic neuropathy, infiltrative disorders, eg, malignancy, amyloidosis and sarcoidosis. Coexistence with other autoimmune diseases like Sjogren syndrome or thyroiditis indicates that there may be immune-mediated destruction of inhibitory oesophageal neurons.

Microscopy

Oesophageal wall is thickened in the distal portion (there is smooth muscle hypertrophy, particularly of inner circular layer); proximal dilated segment is actually thinned out.

Q. Write briefly on gastroesophageal reflux disease (GERD).

Ans. GERD is a chronic diffuse erosive/ulcerative oesophagitis. Normally the oesophageal lining is protected from acids by

1. The abundant submucosal glands in proximal and distal oesophagus (which secrete mucin and bicarbonate)
2. The tone of LES which prevents reflux of acidic gastric contents

Pathogenesis

Both genetic and environmental factors contribute to cause decreased LES pressure which allows reflux.

Predisposing Factors

Pregnancy, ascites, obesity, delayed gastric emptying and peristaltic disorders, eg, scleroderma

Clinical Features

Heartburn, regurgitation, dysphagia/odynophagia, water brash (hypersalivation) and a typical intermittent chest pain

Complications

Ulceration, haematemesis, melena, stricture formation and Barret oesophagus

Diagnosis

X-ray and endoscopy

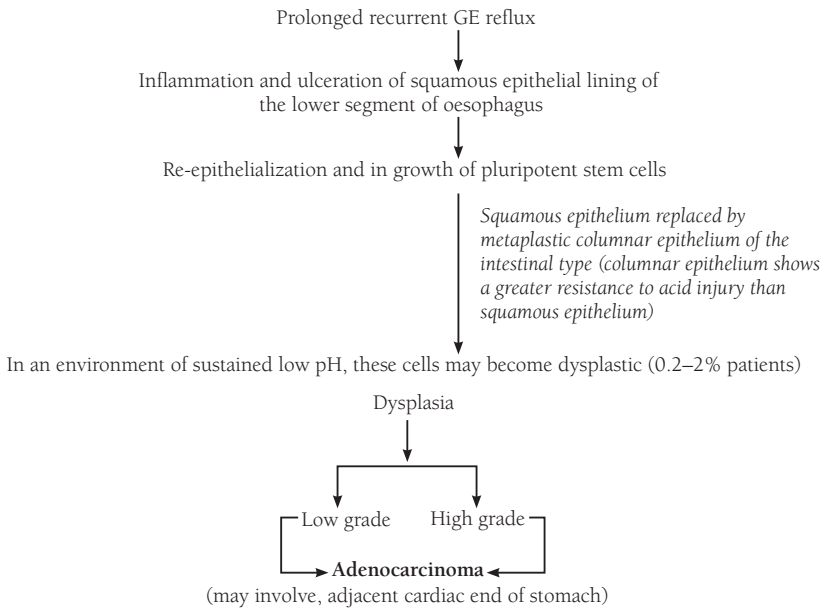
Pathology

- One-third patients have a normal appearing mucosa. Erythema and red streaks are earliest markers of disease followed by erosions and ulcers.
- There may be contact bleeding with endoscope indicating increased friability.
- Dilated vessels and inflammatory cells are present.
- Predominance of eosinophils points to a diagnosis of **'eosinophilic esophagitis'** which is seen in patients with atopy with coexisting atopic dermatitis, rhinitis, asthma and eosinophilia. These patients present with dysphagia and symptoms of food impaction.

Q. Write briefly on Barrett's oesophagus.

Ans. Barrett's oesophagus is a complication of long-standing GE reflux. It is seen between 40 and 60 years of age Males are more commonly affected than females. Hallmark is replacement of distal squamous mucosa by metaplastic columnar epithelium as a response to prolonged injury.

Pathogenesis (Flowchart 14.1)



FLOWCHART 14.1. Pathogenesis and consequences of Barett's oesophagus.

Gross Morphology

Red and velvety mucosa with raised patches, which later form large nodular masses with infiltrative or ulcerative features.

Microscopy

- Most important complication is the development of adenocarcinoma (30–40 folds increased risk).
- Most tumours are mucin-producing glandular tumours.
- Occasional development of SCC, adenosquamous or adenocarcinoid tumours supports the concept that Barrett's epithelium arises from pluripotent cells.

Q. Write briefly on carcinoma oesophagus.

Ans. Age group affected in carcinoma oesophagus is more than 50 years; males are more commonly affected than females.

Predisposing Conditions/Factors

1. **Adenocarcinoma:** Incidence is on the rise in western countries due to rampant obesity which in turn is responsible for increasing the incidence of GERD and Barrett mucosa.
2. **Squamous cell carcinoma:** Most common type worldwide. Predisposing factors include
   (a) Achalasia
   (b) Plummer–Vinson syndrome
   (c) Strictures, diverticulae and webs
   (d) Alcohol, hot and spicy foods, betel chewing, smoking, aflatoxins and silica
   (e) Diet deficient in vitamins A, C and trace elements
   (f) Diet high in nitrosamines

Clinical Features

Dysphagia/odynophagia, weight loss, iron deficiency, haemorrhage and sepsis from the tumour, chest pain and vomiting.

Gross Morphology

60% polypoidal (fungating) lesions, 25% ulcerative and 15% diffuse infiltrating lesions.

Microscopy

- Majority are SCCs which involve the upper thoracic segment (half occurring in middle third of oesophagus).
- May be superficial (carcinoma limited to mucosa and submucosa) or advanced (infiltrating into muscularis propria). Superficial lesions have a much better prognosis.
- Adenocarcinomas and undifferentiated carcinomas are less common.
- Adenocarcinomas usually arise in oesophageal mucous glands or Barrett's oesophagus.
- Visceral metastasis to liver, lung and kidney is early and frequent.
- Overall prognosis is very poor.

STOMACH

Stomach is a saccular organ with a volume of about 1.5 L. It is divided into five anatomic regions, each of which has different histology and functions. These are

1. Cardia: Where the contents of the oesophagus empty into the stomach
2. Fundus: Formed by the upper curvature of the organ
3. Body: Main central dome-shaped part
4. Pylorus: Lower part, which empties the contents of stomach into the small intestine
5. Pyloric sphincter: Stomach demarcated from the duodenum by this muscular sphincter

Layers of Stomach

- Mucosa: Consists of epithelium, lamina propria and a thin layer of smooth muscle labelled muscularis mucosae
- Submucosa: Consists of fibrous connective tissue with the Meissner's plexus
- Muscularis externa: Three layers of smooth muscle, namely:
  - Inner oblique layer
  - Middle circular layer
  - Outer longitudinal layer

  Auerbach's plexus is found between the outer longitudinal layer and middle circular layer.

Normal gastric mucosa has two compartments:

1. Superficial foveolar, which is uniform throughout the stomach.
2. Deeper glandular compartment which has different types of cells found in the different layers of these glands (Table 14.1).

TABLE 14.1. Cells found in different layers of the deeper glandular component of gastric mucosa

Name	Secretory product	Location in stomach
Mucous cells	Mucous and pepsinogen II	Cardiac and pyloric regions
Brightly eosinophilic parietal (oxyntic) cells	Acid and intrinsic factor	Fundic, cardiac and pyloric regions
Basophilic chief (zymogenic cells)	Pepsinogen I and II	Fundic region
Endocrine (APUD) cells	Gastrin, histamine, endorphins, serotonin, cholecystokinin and somatostatin	Fundic, cardiac and pyloric regions

Q. Classify gastritis.

Ans. Gastritis is classified based on

1. The inflammatory cell type and duration:
   (a) Acute gastritis (infiltration by neutrophils)
   (b) Chronic gastritis (infiltration by lymphocytes and plasma cells)
      - *Helicobacter pylori*–induced gastritis
      - Autoimmune gastritis
      - Others
2. The region involved
   (a) Antral gastritis
   (b) Corpus gastritis
   (c) Pan gastritis
3. The presence of premalignant changes
   (a) Nonatrophic
   (b) Atrophic (may progress to carcinoma)

Q. Describe the aetiology, morphology and clinical presentation of acute gastritis.

Ans. Acute transient inflammation of gastric mucosa is labelled acute gastritis.

Aetiology

Acute and chronic gastritis occur when there is a dominance of damaging factors or breakdown of gastroduodenal defence mechanisms.

Clinical Features

- Asymptomatic
- Epigastric pain of variable severity, nausea and vomiting
- Mucosal erosion/ulceration may occur with severe gastritis leading to haemorrhage, haematemesis and melena
- **Gastropathy** is a group of disorders of diverse aetiology (alcohol, NSAIDs, bile, stress) which cause gastric dysfunction and may present like acute gastritis.

Morphology

- In mild gastritis, no significant change is seen.
- Mucosal erosion/ulceration may occur with severe gastritis leading to haemorrhage **(acute erosive gastritis).**

Q. Describe the aetiology, morphology and complications of chronic gastritis.

Ans. Chronic inflammation of gastric mucosa and submucosa results in mucosal atrophy, epithelial metaplasia, dysplasia and predisposition to development of carcinoma without accompanying mucosal erosion.

Aetiology

- ***Infection***:
  - *H. pylori* is a gastric pathogen that has a strong causal association with gastritis and peptic ulcer disease.
  - Chronic infection with this pathogen is known to be associated with gastric adenocarcinoma and low-grade gastric lymphoma.
  - It is a Gram-negative, noninvasive, non-sporing and rod-shaped bacteria.
  - *H. pylori* mediated gastritis is the result of combined influence of bacterial enzymes and toxins with release of toxic chemicals from recruited neutrophils.

- ***Autoimmunity*:** Presence of autoantibodies, to gastric parietal cells, mainly to the acid-producing enzyme H+/K+-ATPase leading to loss of both acid-producing and intrinsic factor-producing cells. The gastric corpus (body) undergoes progressive atrophy. Its sequelae include development of pernicious anaemia, adenocarcinoma and gastric carcinoid.
- ***Toxic substances*:** Alcohol intake and tobacco smoking
- ***Iatrogenic causes*:** Postsurgical (antrectomy and gastroenterostomy)
- ***Radiation exposure***: Radiation-induced gastritis is an infrequent cause of gastrointestinal bleeding.
- ***Infectious granulomatous gastritis*:** Granulomatous gastritis is a rare entity caused by organisms like *M. tuberculosis* and fungi usually in patients who are immunosuppressed.
- ***Chronic reactive chemical gastropathy*:** Gastritis may result from long-term intake of aspirin or NSAIDs. It also develops when bile-containing intestinal contents reflux into the stomach.
- ***Others*:** Amyloidosis and graft versus host reactions

Gross Morphology

Mucosa reddened, coarse with thick rugal folds in early, and thinned with flattened rugal folds in long-standing disease

Microscopic Features

- Lamina propria is infiltrated by chronic inflammatory infiltrate composed of lymphocytes and plasma cells. (Fig. 14.5)
- Intestinal metaplasia is frequently seen
- In long-standing disease due to *H. pylori* as well as autoimmune gastritis, there is loss or atrophy of parietal cells, leading to hypochlorhydria or achlorhydria
- This, in turn, may induce G-cell hyperplasia and hypergastrinaemia.
- *H. pylori,* if present, lies in the superficial mucosal layer among the microvilli of epithelial cells.
- Occasionally, dysplasia may develop.

Clinical Features

- Nausea, vomiting and upper abdominal discomfort
- ***Mild form*:** Hypochlorhydria (not achlorhydria), pernicious anaemia absent and serum gastrin level normal or slightly increased
- ***Severe form*:** Achlorhydria, pernicious anaemia and hypergastrinaemia

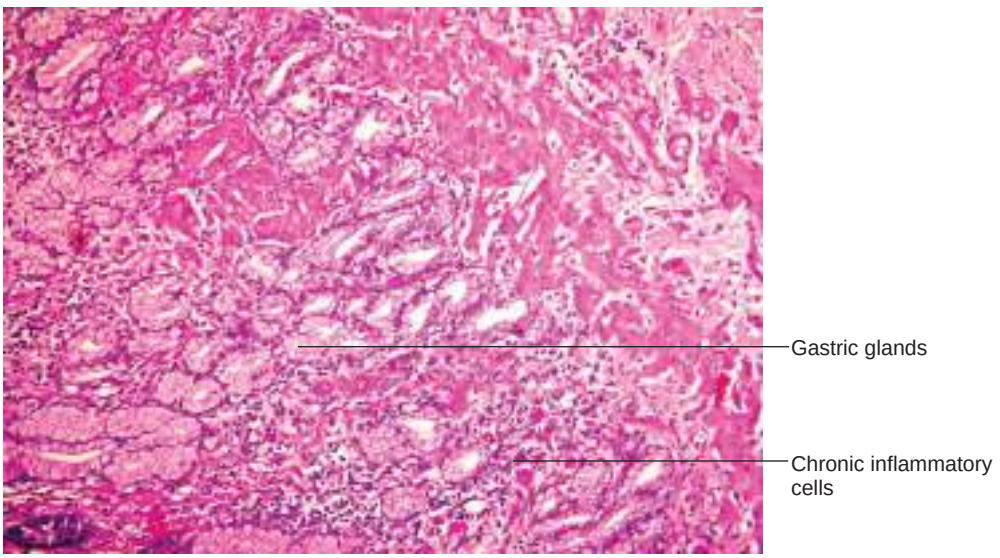


FIGURE 14.5. Gastric mucosa showing chronic nonspecific inflammation (H&E; 200×).

Complications

Peptic ulcer and gastric carcinoma.

Q. Write briefly on the aetiology, pathology and complications of peptic ulcer.

Ans. Peptic ulcer is a chronic (remitting and relapsing), often solitary lesion, present in any part of GIT that is exposed to acid and peptic juices; usually, diagnosed in middle to old age.

Sites (in Decreasing Order)

- First portion of duodenum
- Antral region of stomach (98% of the peptic ulcers present in duodenum or stomach; ratio of duodenal and gastric ulcers is about 4:1)
- Gastroesophageal junction
- Margins of gastrojejunostomy
- Duodenum, stomach and jejunum in Zollinger–Ellison syndrome
- Meckel's diverticulum

Pathogenesis

Peptic ulcer results whenever defence mechanisms of stomach are impaired and/or damaging factors become predominant.

Gastroduodenal defence mechanisms

- Mucous layer on surface
- Bicarbonate secretion into mucosa
- Adequate mucosal blood flow
- Apical surface membrane transport
- Epithelial regenerative capacity
- Prostaglandin secretion

Damaging factors

Gastric acid and peptic enzyme secretion is aggravated by:

- NSAIDs like aspirin (direct mucosal irritation and reduction in prostaglandin and bicarbonate secretion)
- Cigarette smoking and alcohol (impair blood flow and healing capacity of the mucosa)
- Ischaemia and shock (decreased oxygen delivery)
- Iron preparations (direct mucosal damage)
- Viral infections (direct mucosal damage)
- Ageing (reduced mucin and bicarbonate secretion)
- Urease secreting *H. pylori* and gastric injury associated with uraemia (inhibition of gastric bicarbonate transporters by ammonium ions)
- Chemotherapy and chemicals (direct mucosal injury)
- Duodenal-gastric reflux
- Psychological stress
- Hyperkalaemia

Role of H. pylori in peptic ulceration

- It secretes **urease**, **protease** and **phospholipase.**
- Urease generates free ammonia that binds with H+ and decreases acidity; thus, colonization and survival of the organism is favoured.
- Proteases damage the glycoprotein of gastric mucous.
- Phospholipases damage surface epithelial cells and also release leukotrienes and eicosanoids.

- Increases production of proinflammatory cytokines, eg, IL-1, IL-6, TNF and IL-8 (chemotactic to neutrophils).
- May cause thrombotic occlusion causing ischaemia.
- Epithelial injury is induced by VacA (a vacuolating toxin) regulated by CagA (cytotoxin-associated gene).

***H. pylori* is associated with duodenal ulcer in 80–90% patients and gastric ulcer in 60% patients.** Virulence of the infecting strain determines development of peptic ulcer in an individual infected with H. pylori.

Gross Morphology

- Small, round-to-oval, sharply punched-out ulcers, varying in size between 2 and 4 cm.
- Straight wall, mucosa may overhang the base.
- Base—clean and smooth (due to peptic digestion)

Microscopic Features

In an active ulcer, four distinct zones are appreciated (**Askanazy zones**; Fig. 14.6):

1. Zone of fibrinoid necrosis
2. Zone of nonspecific inflammatory infiltrate (predominantly neutrophils)
3. Zone of granulation tissue (proliferating blood vessels, fibroblasts and mononuclear cells)
4. Zone of fibrosis (collagenous or fibrous scar formation)

Complications

- *Bleeding*: Most frequent complication (seen in 15–20% of the patients); may be life threatening, and sometimes, is the first indication of presence of peptic ulcer.
- *Perforation*: Life-threatening late presentation (responsible for two-third of the deaths due to ulcers); may result in acute peritonitis and subphrenic abscess; may involve adjacent organs.
- *Obstruction*: Occurs due to oedema or scarring

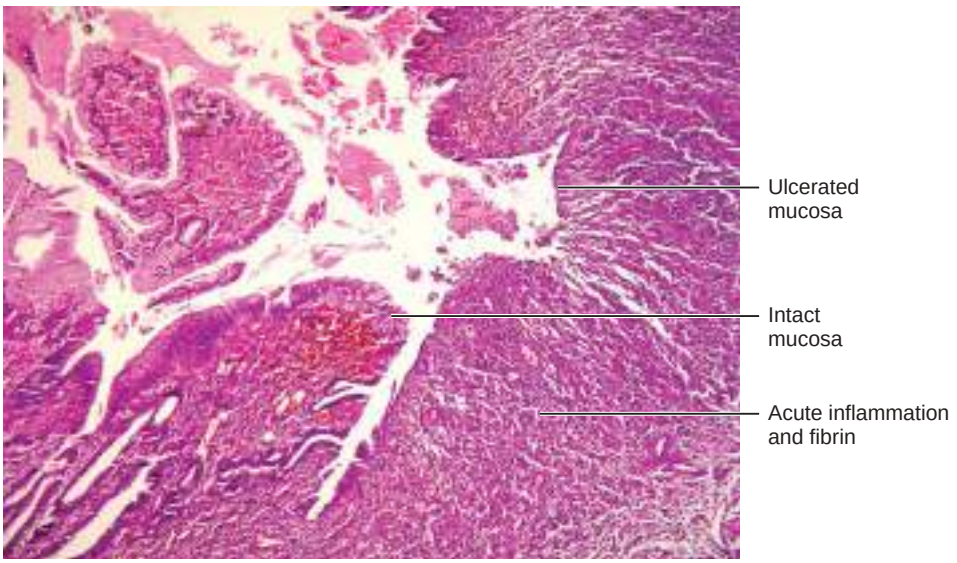


FIGURE 14.6. Section shows both intact (left) and ulcerated mucosa (right). The superficial layer of the ulcer is represented by acute inflammation and fibrin and the base shows inflammatory granulation tissue (H&E; 100×).

Q. Write briefly on hypertrophic gastropathies.

Ans. Hypertrophic gastropathies include

1. **Zollinger–Ellison (ZE) syndrome:**
   - This syndrome presents with hypergastrinaemia due to a gastrinoma (gastrin-producing tumour); usual age of presentation is between 50 and 60 years. The gastrinoma is most frequently found in the duodenum and peripancreatic soft tissues and originates from endocrine cells of both gut and pancreas.
   - Gastrin induces hyperplasia of mucous neck cells (causing diarrhoea), endocrine cells and oxyntic mucosa (causing hypergastrinaemia which in turn induces hypersecretion of gastric acid causing ulcers in the usual sites like stomach and duodenum and also in unusual sites like jejunum).
   - Treatment is surgical resection of the tumour.
2. **Menetrier disease:**
   - Patient aged 30–60 years presents with hypoproteinaemia, weight loss and diarrhoea.
   - There is cerebriform enlargement or hypertrophy of the rugal folds due to epithelial hyperplasia which mainly affects mucous cells in the body and fundus. This is attributed to increased TGF-alfa.
   - Dilated tortuous glands may be seen.
   - Treatment is supportive only (parenteral nutritional supplementation).

Q. Differentiate between duodenal and gastric ulcer.

Ans. Differences between duodenal and gastric ulcer are listed in Table 14.2.

TABLE 14.2. Differences between duodenal and gastric ulcer

Features	Duodenal ulcer	Gastric ulcer
Age	Younger patients (20–50 years)	Older patients (>60 years)
Male-to-female ratio	3:1	1.5–2:1
Incidence	More common	Less common
H. pylori	Stronger association (present in virtually all patients of duodenal ulcer); hypersecretion of acid pepsin is important in pathogenesis	Less strong association (present in 70% of the cases of gastric ulcer); disruption of mucosal barrier is most important pathogenetic factor
Favoured location	First part of duodenum (anterior wall)	Along lesser curvature and pyloric antrum
Acid level	Usually high	Usually normal; increased only if gastrin level is increased
Pain	Relieved after intake of food	Aggravated after intake of food
Night pain	Common	No night pain
Melena	More common	Less common
Vomiting and haematemesis	Less common	More common
Weight loss	No weight loss	Marked weight loss

Q. Differentiate between benign and malignant peptic ulcer.

Ans. Differences between benign and malignant peptic ulcer are listed in Table 14.3.

TABLE 14.3. Differences between benign and malignant peptic ulcer

Features	Benign ulcer	Malignant ulcer
Age	Comparatively younger age	Older age
Sex	Clear-cut male predominance	Slight male predominance
Site	Usually along lesser curvature of pylorus and antrum	Along greater curvature of stomach
Size	Benign ulcers are generally less than 4 cm (however, size is not an absolute criterion for differentiation between benign and malignant ulcers)	Generally more than 4 cm
Ulcer base	Clear; rarely haemorrhagic	Necrotic debris may be present
Mucosal folds	Radiating from the ulcer crater	Interrupted; flattening of the rugae around the ulcer due to infiltration by malignant cells
Margins	No or minimal heaping	Heaping prominent
Barium meal	Sharply punched-out lesion	Irregular lesion

Q. Classify tumours of stomach.

Ans. ***Classification of tumours of stomach***

1. **Nonepithelial/mesenchymal tumours**
   (a) Gastrointestinal stromal tumours (GISTs)
   (b) Leiomyoma and leiomyosarcoma
   (c) Lipoma
   (d) Schwannoma
   (e) Granular cell tumour
   (f) Lymphoma
2. **Epithelial tumours**
   (a) Intraepithelial gastric neoplasia (adenoma)
   (b) Adenocarcinoma (most common malignancy; may be further sub-typed into: papillary, tubular, mucinous, signet ring, undifferentiated and adenosquamous types)
   (c) Small cell carcinoma
   (d) Carcinoid tumour

Q. Write briefly on the aetiopathogenesis, gross and microscopic features of gastric adenocarcinoma.

Predisposing Factors

- ***Dietary factors***
  - Foods containing nitrites or their precursor nitrates
  - Smoked and salted foods, pickled items
  - Less intake of fresh fruits and vegetables
- ***Host factors***
  - *H. pylori* infection and chronic gastritis manifest with multifocal mucosal atrophy (causes hypochlorhydria which favours *H. pylori* colonization) and intestinal metaplasia (predisposes to intestinal type of gastric carcinoma)
  - Partial gastrectomy (reflux of irritant biliary contents and chronic gastritis)
  - Gastric adenomas
  - Cigarette smoking
  - Menetrier disease
- ***Genetic factors***
  - Blood group A
  - Familial gastric cancers are due to mutations in CDH1, which encodes E-cadherin, responsible for the epithelial intercellular adhesion (loss of E-cadherin is usually associated with diffuse gastric cancer).
  - Mutations in β-catenin, microsatellite instability and hypermethylation of several genes like TGFβRII, BAX, IGFIIR and p16INK4α are noted in sporadic intestinal type gastric cancer.

- ***Racial factors***
  More common in Blacks, Americans and Indians
- ***Geographical influence***
  More prevalent in Japan, Finland and Iceland

Location

- Pylorus and antrum (50–60%)
- Cardia (25%)
- Body and fundus (15–25%)

Less curvature is involved more often as compared to greater curvature and most common location is lesser curvature of antropyloric region.

Classification

1. **Microscopic or histological (Lauren) classification**
   (a) Intestinal type (Fig. 14.7)
      (i) Tumour cells form glands resembling colonic adenocarcinoma.
      (ii) Cells have apical mucin vacuoles.
      (iii) Growth is expansile (grows as a cohesive mass along broad fronts).
   (b) Diffuse/gastric type
      (i) No gland formation
      (ii) Cells show signet ring appearance (nucleus pushed to periphery due to presence of large intracytoplasmic mucin vacuole)
      (iii) Scattered individual cells or small cell nests permeate the gastric wall (infiltrative pattern)
2. **Depth of invasion**
   (a) Early gastric carcinoma: Confined to mucosa and submucosa, muscularis propria not infiltrated; may or may not involve perigastric lymph nodes
   (b) Advanced gastric carcinoma: Infiltrates muscularis propria
3. **Gross appearance**
   (a) Exophytic: Polypoid or cauliflower-like tumour mass protruding into lumen
   (b) Flat/depressed/infiltrative: No obvious tumour mass in the mucosa. In the later stages, the infiltration of a part or entire stomach by individual infiltrating tumour cells giving it a 'leather bottle' appearance **(linitis plastica).**
   (c) Excavated: Shallow crater in early stages to large malignant ulcer in advanced lesions

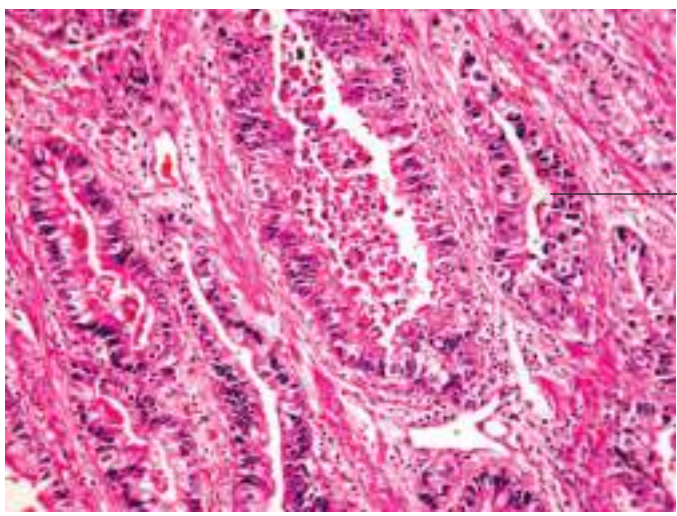


FIGURE 14.7. Well-differentiated intestinal type of adenocarcinoma stomach showing well-formed glands lined by atypical cells with hyperchromatic nuclei infiltrating the gastric wall (H&E; 200×).

Clinical Features

- Abdominal pain
- Anorexia
- Anaemia
- Weight loss
- Vomiting
- Dysphagia due to involvement of cardiac end
- Obstructive symptoms due to involvement of pylorus
- All gastric carcinomas eventually penetrate the serosa to spread to local and distant lymph nodes. Other proposed mechanisms of spread include transperitoneal, lymphatic, haematogenous, via remnants, falciform ligament, etc.
- May frequently metastasize to supraclavicular lymph nodes as the first clinical manifestation of an occult neoplasm **(Virchow lymph node)** or to the periumbilical region to form a subcutaneous nodule **(Sister Mary Joseph nodule)**.
- Another common site for visceral metastasis is bilateral ovaries **(Krukenberg tumour)**. Although uncommon, metastatic adenocarcinoma to the ovary may be seen in association with carcinoma stomach, breast, pancreas and gallbladder.

Q. Write briefly on gastrointestinal stromal tumours (GISTs).

Ans. Gastrointestinal Stromal Tumour (GIST)

- Most common mesenchymal tumour of GIT; most common location is stomach.
- It is male predominant; is seen in the fifth and sixth decades and can present as a triad called **Carney's triad** (gastric GIST, paraganglioma and pulmonary chondroma).
- Origin from interstitial cells of Cajal (which are the pacemaker cells for gut peristalsis and are located in muscular propria).
- Associated with a gain of function mutation of the gene encoding for tyrosine kinase c-kit (receptor for stem cell factor). This leads to constitutional activation of c-KIT which in turn activates the RAS pathway to promote cell proliferation.
- Patient usually presents with an abdominal mass. CT is the best diagnostic modality.
- GISTs may be as large as 30 cm, solitary, well circumscribed, fleshy, submucosal or subserosal masses. When large they may cause ulceration of the overlying mucosa.
- Sections show mainly spindle cells or epithelioid cells or an admixture of the two cell types. Tumour cells express c-KIT (CD117) and CD 34.
- Prognosis of the tumour is dependant on **tumour size** (recurrence and metastasis associated with a size $>$ 5 cm); **mitoses** and **location** (intestinal GISTs are more aggressive than gastric GISTs).

SMALL INTESTINE

- Small intestine varies in length from 4–7 meters. Although it is 4–5 times longer than large intestine, it is referred to as 'small' due to its comparatively smaller diameter.
- The average diameter of the small intestine of an adult human measures approximately 2.5–3 cm, and the large intestine measures about 7.6 cm in diameter.
- It is divided into three structural parts:
  - Duodenum
  - Jejunum
  - Ileum

Duodenum

- Mucosa: Consists of epithelium, lamina propria and a thin muscularis mucosa; epithelium is simple columnar with goblet cells and Paneth cells.
- Submucosa: Composed of Brunner's glands within fibrous connective tissue, which also has Meissner's plexus.
- Muscularis externa: Two layers of smooth muscle, namely, longitudinal and circular. Auerbach's plexus is found between them.

Jejunum and Ileum

- Lack Brunner's glands
- Ileum has Peyer's patches in the lamina propria.
- Small intestine is the site where most nutrients from ingested food are absorbed. It is arranged in folds called plicae circularis which are distinct from rugae, as they are not permanent, allowing distention and contraction of the small intestine.
- From the plicae circularis, project microscopic finger-like villi. Jejunal villi are long; whereas, ileal villi are short.
- The small intestinal mucosa is lined by simple columnar epithelium and the epithelial cells also have finger-like projections known as microvilli.
- The function of the plicae circularis, villi and microvilli is to increase the amount of surface area available for secretion of enzymes and absorption of nutrients.

COLON

- It consists of the ascending, transverse, descending and sigmoid colon. Colon from caecum to the splenic flexure (the junction between the transverse and descending colon) is also known as the right colon. The remainder is known as the left colon.
- There is increase in the thickness of mucosa from caecum to rectum.
- Surface epithelium is composed of absorptive tall columnar epithelium with goblet cells and endocrine cells.
- Columnar cells and goblet cells are present in the ratio of approximately 4:1.
- Paneth cells are most prominent in the caecum and proximal colon (usually confined to crypt bases).

Q. Define and classify malabsorption syndrome.

Ans. Malabsorption syndrome is associated with impaired absorption of nutrients like fat, fat-soluble and other vitamins, proteins, carbohydrates, electrolytes, minerals and water.

Classification

1. Defective intraluminal digestion of fat, proteins and carbohydrates (enzyme deficiency). Normally, the process starts in the oral cavity (saliva) and continues as gastric digestion as well as digestion in the small intestine (aided by pancreatic enzyme secretion and emulsifying action of bile).
   Causes
   - Pancreatic insufficiency (pancreatitis and cystic fibrosis)
   - Zollinger–Ellison syndrome (inactivation of pancreatic enzymes by excess gastric acid secretion)
   - Defective bile secretion
2. Defective mucosal absorption of fat, proteins, carbohydrates, water and minerals.
   Causes
   - Primary mucosal cell abnormalities: Defective terminal digestion and defective epithelial transport, eg, disaccharidase deficiency (lactose intolerance) and bacterial overgrowth with brush border damage.
   - Reduced small intestinal surface area: Crohn disease, celiac sprue and surgery
   - Lymphatic obstruction: Lymphoma and tuberculosis
   - Infections like tropical sprue, Whipple disease and parasitic infestation

Clinical Features

Depend on the type of malabsorption; signs and symptoms may be related to specific nutrient deficiency or may be due to generalized deficiency and are as follows:

- Passage of bulky, frothy, greasy, yellow or grey stools, abdominal distension and flatus
- Weight loss and muscle wasting
- Anaemia from iron, pyridoxine, folate or vitamin B_{12} deficiency
- Bleeding (petechiae and purpura) due to vitamin K deficiency

- Oedema due to protein deficiency
- Dermatitis, mucositis and hyperkeratosis due to vitamin A, zinc, essential fatty acids and niacin deficiency
- Osteopenia and tetany due to defective calcium absorption
- Amenorrhea, impotence and infertility from generalized malnutrition
- Hyperparathyroidism due to protracted calcium and vitamin deficiency

Q. Differentiate between celiac (nontropical) sprue and environmental or tropical enteropathy.

Ans. Differences between celiac and tropical sprue are listed in Table 14.4.

TABLE 14.4. Differences between celiac and tropical sprue

Features	Celiac sprue	Environmental or tropical enteropathy (tropical sprue)
Other names	Gluten-sensitive enteropathy, nontropical sprue	Post-infectious sprue; may occur in epidemic or endemic forms
Pathogenesis	Immune-mediated disease due to sensitivity to gluten and related proteins (water insoluble gliadin) present in wheat, oat, barley and rye. No organism implicated. Gladden peptides induce secretion of IL 15 which activates CD8+ intraepithelial lymphocytes. These lymphocytes express NKG2D, a natural killer cell marker and receptor for MHC class I polypeptide-related sequence (MIC-A). Epithelial cells which express surface MIC are recognized and attacked by NKG2D expressing lymphocytes.	Infectious disease. Occurs exclusively in patients living in or visiting the tropics. No specific causal agent implicated. Enterotoxigenic bacterial (cyclospora and *E. coli*) overgrowth is found
Genetic predisposition	HLA (DQ2 and DQ8) association accounts for almost half of the genetic component of celiac disease. The remaining genetic factors include polymorphisms of immune regulatory genes like IL-2 and IL-21	None
Distribution	Affects mainly the proximal part of small intestine (higher gluten exposure than distal part)	Affects the distal small bowel
Associated clinical conditions	Dermatitis herpetiformis and neurological disorders	Frequent folate or vitamin B_{12} deficiency (due to involvement of distal small bowel) leading to atypical enlargement of nuclei of epithelial cells (megaloblastic change)
Secondary malignancy	Intestinal lymphoma, small intestinal adenocarcinoma and oesophageal squamous cell carcinoma	No such predisposition
Treatment	Gluten-restricted diet	Broad-spectrum antibiotics

Q. Enumerate the morphologic features of celiac sprue.

Ans. Morphological Features

- Diffuse enteritis with atrophy or total loss of villi.
- Vacuolar degeneration of surface epithelium, loss of microvilli and increased number of intraepithelial CD8+ T lymphocytes.
- In an attempt to maintain mucosal thickness, crypts become hyperplastic, elongated and tortuous and also show increased mitotic figures.

- Plasma cells, lymphocytes, macrophages, and mast cell infiltration in lamina propria

Note: The above pathological findings are characteristic of celiac sprue but nonspecific and can be seen in tropical sprue as well. Mucosal histology reverts to normal after excluding gluten from the diet.

Q. Enumerate the ulceroinflammatory diseases of small and large intestine.

Ans. Small intestine

- Crohn disease
- Typhoid ulcer
- Tuberculous ulcer
- Ulcers due to *Campylobacter spp.*
- Drug-induced ulcers

Large intestine

- Ulcerative colitis
- Shigella-induced ulcers
- Ulcers due to *Campylobacter spp.*
- Amoebic ulcers

Q. Write briefly on the pathology and complications of intestinal tuberculosis.

Ans. Intestinal tuberculosis may be **primary** (caused by *Mycobacterium bovis* ingested via unpasteurized milk) or **secondary** (in a patient of active pulmonary tuberculosis, swallowing of coughed up material results in secondary tuberculosis of intestine).

Salient Features

- It mainly occurs in terminal ileum; colon is rarely involved.
- Primary tuberculosis of intestine mainly involves mesenteric lymph nodes, which are enlarged, caseous and matted, and usually heal by fibrosis and calcification.
- Intestinal lesions are more prominent than nodal lesions in secondary tuberculosis. Lesion starts as a small ulcerative lesion, which progressively enlarges to form a large transverse ulcer, perpendicular to the long axis of the bowel (as it spreads through lacteals or lymphatics, which are transversely oriented). Serosa may also exhibit tubercles.
- Hyperplastic caecal tuberculosis is a variant of secondary intestinal tuberculosis involving caecum (sometimes ascending colon), which is commonly palpable as a lump (called hyperplastic because the tuberculous granulation tissue formed in this lesion masquerades as a lump).

Complications

- Tuberculous peritonitis may occur as a part of disseminated tuberculosis or result from a tuberculous lesion in close proximity to the peritoneum. It may manifest as effusion or as fibrosis (doughy abdomen).
- Fibrous stricture occurs due to transverse or circumferential ulceration and can lead to intestinal obstruction.

Q. Differentiate between tuberculous and typhoid ulcer of intestine.

Ans. Differences between tuberculous and typhoid ulcer are listed in Table 14.5.

TABLE 14.5. Differences between tuberculous and typhoid ulcer

Features	Tuberculous ulcer	Typhoid ulcer
Causative organism	*Mycobacterium tuberculosis*	*Salmonella typhi*
Site	Anywhere in small intestine; most common in terminal ileum and caecum, rarely colon	Most common in terminal ileum (Peyer's patches); may occur in jejunum
Orientation of the ulcer	Perpendicular to long axis of bowel (transverse ulcer) due to spread by lacteals (lymphatics)	Parallel to long axis of bowel (longitudinal ulcer) due to involvement of Peyer's patches
Microscopic features	Epithelioid cell granulomas with or without caseous necrosis	Lymphoplasmacytic infiltrate with histiocytes some of which show erythrophagocytosis
Fibrosis and stricture formation	Common; intestinal tuberculosis may present with subacute or acute intestinal obstruction	Rare
Perforation	May be present	Common
Bleeding	Absent	Present

Q. Define inflammatory bowel disease (IBD). Write briefly on its aetiopathogenesis.

Ans. IBD is a chronic relapsing inflammatory disorder of unknown origin, which results from an abnormal immune response to normal flora of gut/self-antigens, in genetically susceptible individuals.

Pathogenesis of IBD involves genetic susceptibility, immune dysregulation and triggering by microbial flora.

Genetic Predisposition

- IBD is linked to specific HLA types; (ulcerative colitis with HLADRB1 and HLADR7 and Crohn disease with HLADQ4).
- Association with non-HLA genes, namely, **NOD2** (nucleotide-binding oligomerization domain-2) and a mutant form of **IL23**, is well known.
- NOD2 is an intracellular receptor for **muramyl dipeptidase**, a component of the cell wall in many bacteria, which plays an important role in host responses to these bacteria. It is expressed in **Paneth cells.**
- The mutant form is defective in its response to the bacteria, thus allowing chronic infection to be established in the intestine and promoting inflammatory reactions.
- Alternately, the disease-associated mutant form may promote excessive host response to the intestinal bacteria.
- IL-23 promotes production of IL17 by T cells and IL17 has been implicated in inflammatory reactions seen in IBD and other chronic diseases.

Immunological Reactions

- Immune reactions may be directed against self-antigens of the intestine or bacterial antigens.
- Primary damaging cells appear to be CD4+ T cells.
- Tissue inflammation may be the result of secretion of IL17 by a recently discovered subset of CD4+ T cells called **T_H17 subset**.
- **TNF** may play an important role in the pathogenesis of Crohn disease (proven by the fact that TNF antagonists effectively control the disease).

Epithelial Defects

- Presence of defects in intestinal epithelial tight junction barrier function (associated with NOD2 polymorphisms).
- Mutation of the organic cation transporter SLC22A4 in Crohn disease leading to the defective transepithelial transport.
- Defects in extracellular barrier formed by secreted mucin.
- Abnormality in Paneth cell granules, which contains antibacterial peptides called defensins due to ATG16L1 mutations, is implicated in IBD. It is thought that defective epithelial anti-microbial function may contribute to the genesis of IBD.

Microbial Factors

Microbes provide an antigenic trigger to a basically dysregulated immune system.

Inflammation

Inflammation is the final common pathway for pathogenesis of IBD. It induces

- Impaired integrity of mucosal–epithelial barrier
- Loss of surface epithelial cell absorptive function

Q. Outline the clinical features and morphology of Crohn disease.

Ans. Crohn disease (also called terminal ileitis, regional enteritis or granulomatous colitis) is a systemic inflammatory disease, which predominantly affects GIT (mainly terminal ileum, ileocaecal valve and caecum) and has the following characteristic features:

- Sharply delimited and typically transmural involvement of bowel
- Presence of noncaseating granulomas
- Fissuring with formation of fistulas

Clinical Features

- May affect any age, but major peaks in the second and third decades of life
- Presents with recurrent episodes of diarrhoea, crampy abdominal pain, fever and melena
- Remissions and relapses are common.
- Patients may develop **malabsorption, fistula formation** and **intestinal stricture** or **obstruction. Fistula may form to other loops of bowel, urinary bladder, vagina and perianal skin.**
- **Extraintestinal manifestations** include uveitis, sacroiliitis, migratory polyarthritis, erythema nodosum, bile duct inflammatory disorder, obstructive uropathy and nephrolithiasis.

Gross Morphology

- Serosa is dull and granular with creeping fat appearance.
- Mesentery is thickened, edematous or fibrotic.
- Intestinal wall is rubbery and thick due to oedema and inflammation in the early stages and fibrosis and hypertrophy of muscularis propria in the later stages.
- Lumen is narrowed **(string sign on X-ray).**
- **Skip lesions are characteristic** (sharp demarcation of the involved segment from the uninvolved).
- Aphthous linear ulcers **(cobblestone appearance),** fistula or sinus tract formation, depending on the stage of the disease, may be seen.

Microscopy

- Active Crohn disease shows abundant neutrophils in the lamina propria and crypts which damage the crypt epithelium and form crypt abscesses. Ulceration is frequent.
- Repeated cycles of crypt damage and regeneration lead to architectural distortion of the mucosa. Branching crypts with abnormal shapes replace the normally straight and parallel crypts.
- Transmural inflammation affecting all layers can be demonstrated.
- Noncaseating granulomas and fibrosis are common. Granulomas can also be seen in the mesenteric lymph nodes. Cutaneous nodules form which also show noncaseating granulomas (earlier labelled metastatic Crohn disease).

Q. Describe the clinical features, morphology and complications of ulcerative colitis.

Ans. Ulcerative colitis is an ulceroinflammatory disease limited to colon. It usually affects only the mucosa and submucosa except in very severe forms. It peaks between 20 and 25 years and is more common in females.

Gross Morphology

- The lesion extends in a retrograde and continuous fashion from rectum to proximal parts of colon; **no skip lesions are seen.** Involvement of a few centimetres of ileum when the entire colon is involved can be seen and is termed '**backwash ileitis**'.
- The mucosa is red, granular and friable mucosa which bleeds easily
- Broad-based mucosal ulcers; aligned along the long axis of the colon and pseudopolyps (due to bulging of regenerating mucosa) are a common sight
- No mural thickening is seen the serosa is normal

Microscopy

- Mucosal inflammation, chronic mucosal damage and ulceration (ulcer limited to mucosa and submucosa)
- A diffuse, predominantly mononuclear infiltrate in lamina propria
- Crypt abscesses (due to neutrophilic infiltration of crypts in active stage)
- Even after healing, mucosal architectural disarray, colonic gland atrophy and submucosal fibrosis may be seen.
- Epithelial dysplasia is common.

Complications

- Toxic megacolon (caused by neuromuscular shutdown due to damage to muscularis propria and neural plexus)
- Perianal fistula
- Development of colonic carcinoma
- Bleeding
- Perforation (damage to muscularis propria leads to perforation and pericolonic abscess formation)

Q. Differentiate between Crohn disease and ulcerative colitis.

Ans. Differences between Crohn disease and ulcerative colitis are summarized in the Table 14.6.

TABLE 14.6. Differences between Crohn disease and ulcerative colitis

Features	Crohn disease	Ulcerative colitis
Gross features		
Bowel region affected	Ileum, sometimes colon (may involve any part of GIT)	Colon
Pattern of distribution	Skip lesions	Diffuse, continuous involvement
Stricture formation	Early	Late, uncommon
Intestinal wall	Thickened	Thinned out
Intestinal dilatation	Absent	Present
Progression	Antegrade	Retrograde
Microscopic features		
Ulcers	Deep linear	Superficial
Pseudo polyps	Absent	Present
Lymphoid reaction	Marked	Mild
Fibrosis	Marked	Mild
Serositis	Marked	Absent or mild
Granulomas	Present in 50% of the cases	Absent
Fistula/sinus	Present	Absent
Clinical features		
Fat/vitamin malabsorption	Present	Absent
Malignant potential	Less	More
Response to surgery	Poor	Good

Q. Write briefly on amoebic colitis. Differentiate between amoebic and ulcerative colitis.

Ans. Salient Features of Amoebic Colitis is caused by the protozoan *Entamoeba histolytica.*

- *Amoebic colitis is caused by the protozoan Entamoeba histolytica.*
- The life cycle of ***E. histolytica*** has the following stages:
  1. Trophozoite stage: Spherical to oval trophozoites can be demonstrated in the stool of patients who exhibit acute symptoms.
  2. Precyst stage: The trophozoite converts into a precyst form in the colon of the patient.
  3. Cyst stage: Amoebic cysts have a thick chitinous wall and four nuclei. Infection occurs by the faecal route due to ingestion of food contaminated with the faeces containing the cysts.
- The cysts are resistant to gastric acid and are passed as it is to the colon where they colonize the epithelial surface to release trophozoites. Most frequent location of colonization is caecum and ascending colon.
- Trophozoites produce a lytic substance which aids in invasion of the crypts. They then burrow laterally into the lamina propria to form a superficial **flask-shaped ulcer** with a narrow neck and wide base.
- Trophozoites may reach the liver by invading blood vessels to produce an **amoebic liver abscess** in about 40% cases.
- Clinically patient in the **acute stage** presents with abdominal pain and bloody diarrhoea. **Amoebic liver abscess** typically manifests with right upper quadrant pain, low-grade fever and weight loss.
- Trophozoites can microscopically be demonstrated in the surface of the ulcer which shows both acute and chronic inflammation.
- Thickening of the intestinal wall with napkin ring–like constriction (**ameboma**) may occasionally occur and can be confused with malignancy.
- Diagnosis is based on stool examination, serology and radiology.

Differences between amoebic and ulcerative colitis are listed in Table 14.7.

TABLE 14.7. Differences between amoebic and ulcerative colitis

Features	Amoebic colitis	Ulcerative colitis
Cause	Infective; caused by *Entamoeba histolytica*	Unknown; may result from dysregulated immune responses, in genetically susceptible individuals
Pathogenesis	Transmission by faecal-oral route (infection is spread through ingestion of cyst form of the parasite, a resistant structure that is found in stools)	Genetic predisposition with immunologic dysregulation
Distribution	Localized to caecum and ascending colon, sigmoid, rectum and appendix in decreasing order. In severe cases, entire large intestine may be involved	Involves rectum and extends proximally to involve whole colon in severe cases
Ulcer	Pin-head to large-sized ulcers seen. Muscularis propria acts as a barrier to trophozoites. The ulcer fans out laterally just above the muscularis propria, to form discrete flask-shaped ulcers (narrow neck and broad base). Intervening mucosa is normal	• Broad-based ulcers with continuous involvement; no intervening normal mucosa. • Usually superficial: limited to mucosa and submucosa
Morphology	Liquefactive necrosis; few inflammatory cells; mainly neutrophils	Diffuse mononuclear infiltrate, crypt abscesses
Pseudopolyps	Absent	Present
Risk of cancer	Nil	Present

Q. Classify polyps of the intestine and describe their clinicopathological features.

Ans. Classification of Polyps of the Intestine

1. **Nonneoplastic polyps,** which include inflammatory, hamartomatous and hyperplastic polyps.
   (a) Inflammatory polyps
      (i) Present with a clinical triad of rectal bleeding, mucous discharge and a lesion in the anterior rectal wall.
      (ii) The lesion is due to an abnormal anorectal sphincter that leads to recurrent abrasion and ulceration of the overlying rectal mucosa.
      (iii) Recurrent injury and healing causes some degree of mucosal prolapse and formation of the inflammatory polyp.
      (iv) Microscopically, the polyp shows epithelial and fibromuscular hyperplasia and a mixed inflammatory infiltrate in the lamina propria.
   (b) Hamartomatous polyps: Occur sporadically and as part of genetic or acquired syndromes, examples are:
      (i) Juvenile polyps
         - Focal malformations of mucosal epithelium and lamina propria, which usually occur in children less than 5 years of age
         - Majority occurs in rectum and present with rectal bleeding or prolapse
         - May be sporadic or syndromic
         - Sporadic juvenile polyps are usually solitary lesions and are called retention polyps.
         - Individuals with autosomal dominant inheritance have 3–100 or more polyps. Most common mutation is of SMAD4 (which encodes an intermediate in TGF-β pathway). The juvenile polyposis syndrome is associated with a higher risk of colonic adenocarcinoma.
         - Microscopically, cystically dilated glands filled with mucin and inflammatory cells are seen in a background of lamina propria with mixed inflammation.
      (ii) Peutz–Jeghers polyps
         - Peutz–Jeghers polyps are hamartomatous polyps seen in the small intestine, colon and stomach that occur as part of the rare autosomal dominant syndrome called Peutz–Jeghers syndrome.

- Also seen in this syndrome is melanotic mucosal and cutaneous pigmentation and increased risk of several malignancies including cancer of colon, pancreas, breast, lung, ovaries, uterus and testicles. It is caused by a germline mutation in LKB1/STK11 gene that encodes a serine/threonine protein kinases.
- Present as large and pedunculated polyps with a lobulated appearance
- Histologically characterized by extensive connective tissue and smooth muscle arborization (intermixing) throughout the polyp; the glands being lined by normal looking intestinal epithelium

(iii) Cowden syndrome
- Hamartomatous polyps in GIT associated with an increased risk of neoplasms of thyroid, breast, uterus and skin
- Caused by a germline mutation in PTEN (phosphatase and tensin homologue) tumour suppressor gene
- PTEN encodes a phosphatase that acts as an inhibitor of signals from several tyrosine kinase receptors and favours apoptosis through the BAD/BCL2 pathways

(iv) Cronkhite–Canada syndrome
- Nonhereditary polyposis seen in individuals over 50 years who present with diarrhoea, weight loss, abdominal pain and weakness
- Hamartomatous polyps are seen in stomach, small intestine and colorectum.
- Polyps are histologically similar to juvenile polyps.
- Intervening nonpolypoidal mucosa also shows crypt dilatation, oedema and inflammation in the lamina propria.
- Other manifestations include nail atrophy or splitting, hair loss and hypo- and hyperpigmentation of the skin.

(c) Hyperplastic polyps
(i) Epithelial proliferations that are thought to result from delayed shedding of surface epithelial cells lead to piling of goblet and absorptive cells.
(ii) Hyperplastic polyps do not have a malignant potential.
(iii) The crowding gives rise to a serrated surface (histological hallmark).
(iv) A sessile serrated adenoma, which is histologically similar but has malignant potential, needs to be differentiated from a hyperplastic polyp.
(v) Classically less than 5 mm and seen in left colon.
(vi) May be single or multiple

2. **Neoplastic polyps (adenomas of the small and large intestine)**
(a) Are variable in size and may be pedunculated or sessile; show a progressive increase in incidence with increasing age (peak incidence after 60 years)
(b) Familial predisposition present; males and females are equally affected
(c) All adenomas are a result of proliferative epithelial dysplasia and may give rise to invasive carcinomas
(d) Adenomas are classified into four types based on epithelial architecture:
(i) Tubular adenomas
(ii) Villous adenomas
(iii) Tubulovillous adenomas
(iv) Sessile serrated adenomas
(e) Malignant transformation depends on polyp size, histological architecture and severity of epithelial dysplasia. Villous adenomas greater than 4 cm in diameter are likely to undergo malignant transformation.

Tubular adenomas

- May arise anywhere in the colon; about half are found in the rectosigmoid
- May be solitary or multiple
- Large adenomas usually have a slender stalk 1–2 cm long with a raspberry-like head

Histology

- Stalk covered by normal colonic mucosa whereas the head is composed of branching glands lined by dysplastic epithelium, which may or may not be mucin secreting.
- All degrees of atypia may be encountered. Cancer may be limited to mucosa (intramucosal carcinoma) or be frankly invasive, extending into the submucosa of the stalk.

Villous adenomas

- Finger-like polyps, which are larger than most epithelial polyps
- Usually seen in the rectosigmoid; generally, sessile and velvety or cauliflower-like

Histology

- Filiform extensions of mucosa covered by dysplastic epithelium
- All degrees of dysplasia may be encountered
- Invasive carcinoma is seen in about 40% of these lesions

Tubulovillous adenomas

- Show an admixture of tubular and villous areas
- They are intermediate between tubular and villous adenomas in terms of their histology and behaviour

Sessile serrated adenomas

- Premalignant sessile lesions of colon
- Overlap histologically with hyperplastic polyps but unlike hyperplastic polyps have a serrated architecture through the entire length of the gland (in hyperplastic polyps the serrated architecture is limited to the surface of the gland)

Q. Differentiate between tubular and villous adenomas.

Ans. Differences between tubular and villous adenomas are listed in Table 14.8.

TABLE 14.8. Differences between tubular and villous adenomas

Features	Tubular adenoma	Villous adenoma
Architecture	>75% tubular architecture	>50% villous architecture
Incidence	90–95% (most common)	1% of all adenomas
Distribution	90% cases in colon	More in rectum and rectosigmoid
Age	Early	Late
Gross	Small; pedunculated	Large, sessile or cauliflower-like
Histology	Stalk has fibromuscular tissue and prominent blood vessels; covered by non-neoplastic mucosa. Head region shows tubule-like structures lined by dysplastic cells (instead of the normal mucin-secreting colonic mucosa)	Villiform mucosa covered by dysplastic, disorderly columnar epithelium
Risk of malignancy	Low	High

Q. Write briefly on hereditary cancer syndromes of colon.

Ans. Gastrointestinal polyps can develop as sporadic lesions or as part of hereditary polyposis syndromes. The most common colonic cancer-associated syndromes include

1. **Familial adenomatous polyposis (FAP):**
   - This is an autosomal dominant disorder with a genetic defect localized to the APC gene on chromosome 5q21.
   - Patients with FAP typically develop a large number of polyps, which carpet the entire colon.

- A minimum number of 100 are required for the diagnosis.
- Adenomas may also be present in other parts of the intestine, eg, duodenum.
- Polyps manifest as early as adolescence or early adulthood.
- Conversion to colonic cancer is 100% by middle age.
- Specific APC mutations are also seen in variants such as **Gardner syndrome** (osteoma of mandible, skull and long bones, epidermal cysts, desmoid tumours and thyroid tumours) and **Turcot syndrome** (intestinal adenomas and tumours of central nervous system).
- The morphology of the polyps is same as any sporadic adenoma.

2. **Hereditary Nonpolyposis Colorectal Cancer (HNPCC)** or **Lynch Syndrome:**
   - HNPCC is an autosomal dominant condition in which patients have an earlier onset of colorectal cancer as compared to sporadic cases. The cancer is usually found in right colon and is nonaggressive despite being mucinous on histology.
   - Though the name suggests absence of adenomas; adenomas do develop in HNPCC, although few.
   - HNPCC is associated with germline mutations in DNA mismatch repair (MMR) genes which encode enzymes responsible for repair of sequence errors which may occur during DNA replication. An important diagnostic feature of MMR-deficient tumours is the high rate of mutations that accumulate in repetitive nucleotide regions and these mutations are known as microsatellite instability (MSI).
   - Extracolonic cancers associated with HNPCC include: cancers of stomach, small intestine, endometrium, ovary and urinary bladder.

Q. Write briefly on colorectal carcinogenesis/adenoma–carcinoma sequence.

Ans. The following are the salient features of colorectal cancer:

Aetiology

Dietary factors

- High content of refined carbohydrate and low content of dietary fibre reduces bulk, increases transit time, thereby increasing the duration of exposure of colonic epithelium to possible carcinogens in diet and altering the normal intestinal bacterial flora.
- Intake of red meat (high cholesterol and hence high bile acid secretion, whose bacterial byproducts in the colon are considered to be irritants)
- Decreased intake of protective micronutrients (less vitamins A, C and E)

Geographical Variation

More common in North America and Northern Europe rather than South America, Africa and Asia

Familial History

Only in 1–3% cases; most cases are sporadic

Previous Bowel Conditions

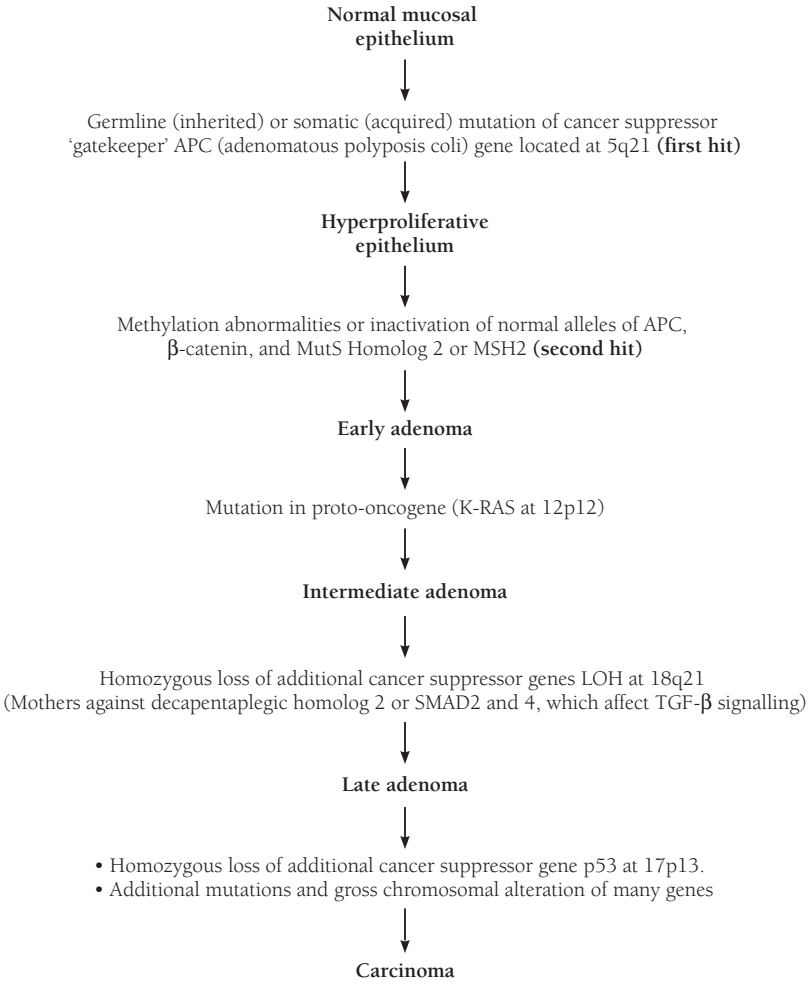
Inflammatory bowel disease, adenomas and diverticular diseases

It has been noted that aspirin and other NSAIDs may have a protective role in colorectal carcinogenesis as they inhibit cyclooxygenase-2 (COX-2) which is expressed in 90% colorectal cancers. COX-2 is instrumental in production of PGE-2 which is thought to be responsible for epithelial proliferation.

Two distinct molecular pathways have been implicated in colonic cancer and they are

1. **Adenoma-carcinoma sequence (APC β-catenin pathway; Flowchart 14.2):**
   - This pathway is responsible for 80% sporadic colonic cancers and involves a series of genetic alterations accompanying the progressive conversion of normal mucosa to malignancy.

- APC is the chief negative regulator of β-catenin, which in turn forms a part of the Wnt signalling pathway.
- APC protein binds to and degrades β-catenin.
- The latter accumulates if there is loss of APC and moves to the nucleus where it complexes with the DNA-binding factor TCF to induce transcription of genes like MYC and Cyclin D1, which promote cellular proliferation.



FLOWCHART 14.2. Adenoma–carcinoma sequence (APC β-catenin pathway).

2. Microsatellite instability pathway (defective DNA repair):

(a) Microsatellites are repeated sequences of 1–6 nucleotides in the genome. They may undergo insertion or deletion of bases during normal cellular replication and these are corrected by DNA mismatch repair (MMR) genes.

(b) Deficiency in cellular MMR leads to widespread mutagenesis and neoplastic development.

(c) An important diagnostic feature of MMR-deficient tumours is the high rate of mutations that accumulate in repetitive nucleotide regions and these mutations are known as microsatellite instability (MSI).

(d) A standard panel of markers to test for MSI in tumours has been recommended and efficiently separates tumours into those with high, low or no microsatellite instability (MSI-H, MSI-L or MSS).

Gross Morphology

Can be found anywhere in the colon and are typically seen as exophytic polypoid (right-side colon) or annular constricting (left-side colon) growths

Microscopic Features

- Ninety-eight percent of all colonic cancers are adenocarcinomas, which vary in differentiation from well differentiated (Fig. 14.8) to poorly differentiated anaplastic tumours.
- Mucin-producing tumours have a poorer prognosis (mucin facilitates spread of tumours as it dissects through the gut wall).
- Signet ring appearance of tumour cells and endocrine differentiation may be seen.
- Anal carcinomas are usually squamous in origin.

Spread of Tumour

- Direct spread
- Lymphatic spread to local lymph nodes, regional and distant lymph node groups
- Haematogenous spread to liver, lungs, brain, bones and ovaries

Prognosis

Most important prognostic criteria for colorectal carcinoma are

1. Depth of invasion (invasion into muscular propria is associated with an adverse prognosis)
2. Presence or absence of lymph node metastasis (lymph node metastasis reduces the survival rate)
3. Poorly differentiated/mucinous tutors are associated with a bad prognosis

The earlier used Dukes and Kirklin and Astler–Coller staging systems have been replaced by TNM and American Joint Committee on Cancer (AJCC) staging systems.

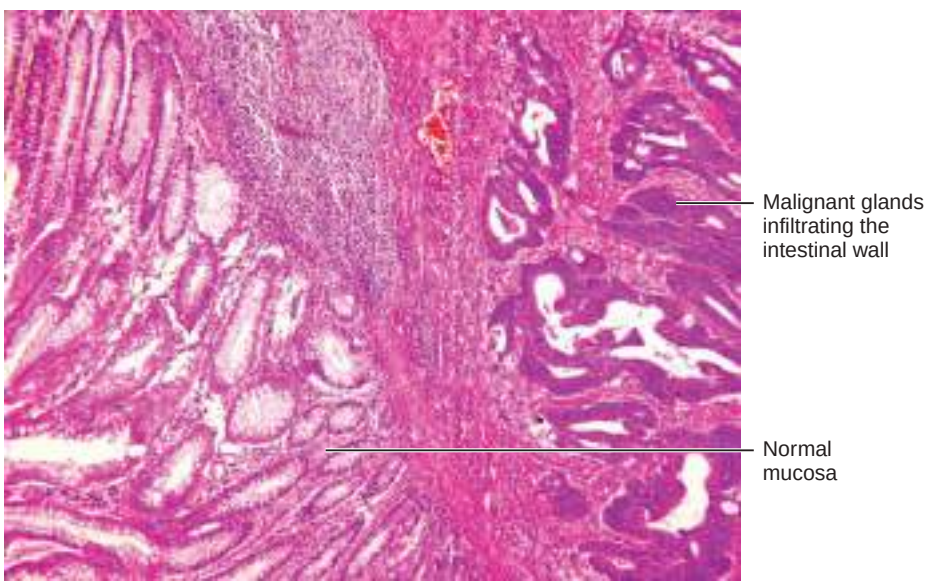


FIGURE 14.8. Section from adenocarcinoma colon showing normal mucosa (left) and mucosa showing malignant change (right). Well-formed glands lined by atypical glandular epithelium infiltrating the intestinal wall are seen in the right side of the section (H&E; 100×).

Q. Enumerate the various modalities for diagnosing colorectal carcinoma.

Ans. Modalities for diagnosing colorectal carcinoma:

1. Digital rectal examination
2. Testing for occult blood loss
3. Double contrast barium enema (apple core appearance), sigmoidoscopy or colonoscopy and endoscopy directed biopsy
4. Computed tomography and other radiographic techniques to look for the primary as well as the spread
5. Serum markers like CEA (has little diagnostic value as levels become significantly elevated only after the tumour has achieved a considerable size; CEA levels also elevated in carcinoma of lung, breast, ovary, urinary bladder and prostate and nonneoplastic disorders like alcoholic cirrhosis, pancreatitis and ulcerative colitis)
6. Molecular detection of APC mutations in epithelial cells from stool is being considered as a diagnostic tool

Q. Differentiate between right-sided and left-sided colonic carcinoma.

Ans. Differences between right-sided and left-sided colonic carcinoma are listed in Table 14.9.

TABLE 14.9. Differences between right-sided and left-sided colonic carcinoma

Features	Right-sided colonic carcinoma	Left-sided colonic carcinoma
Site	Caecum and ascending colon	Descending colon and sigmoid
Gross appearance	Fungating polypoid carcinoma. Large cauliflower-like soft friable mass projecting into lumen	Ulcerative or ulceroinfiltrative lesions producing a napkin ring constriction (annular ring). May show central ulceration with slightly elevated margins
Clinical features	Bleed readily; fatigue, weakness, iron deficiency anaemia. Obstructive symptoms less common due to a larger area available for the tumour to expand	Occult bleeding, change in bowel habits, crampy lower left quadrant discomfort, constipation and obstructive symptoms more prominent
Diagnosis	Late	Early (due to early onset of obstructive symptoms)

Q. Describe the clinicopathological features of carcinoid tumour of GIT.

Ans. Salient Features of Carcinoid Tumour of GIT

- Derived from cells of neuroendocrine origin, which are normally present throughout the GI mucosa
- Constitute about 2% of colorectal malignancies and almost half of the small intestinal malignant tumours
- Release peptide and nonpeptide hormones, which are responsible for their clinical manifestations
- Usually arise in the pancreas, peripancreatic tissue, lungs, biliary tree and liver. In the GIT, appendix is the most common site followed by ileum, rectum, stomach and colon.
- No age is exempt, peak incidence during sixth decade
- Cut surface is solid and yellow-tan.
- The tumour cells have argentaffin granules which stain positive with silver stains.
- Carcinoids are slow-growing tumours with different characteristics and growth patterns and can be subdivided based on the following features:
  - Growth pattern (trabecular, glandular, undifferentiated and mixed)

- Hormone produced (bradykinin, serotonin, histamine and prostaglandins)
- Site of origin: foregut (pancreas, stomach and duodenum), midgut (jejunum, ileum, appendix and ascending colon) or hind gut (transverse colon, descending colon and rectum)

Gross Morphology

- Small button-like submucosal elevation with intact or ulcerated overlying mucosa
- Ileal and gastric carcinoids are multiple, whereas appendiceal carcinoids are solitary and usually involve the tip of the organ

Microscopic Features

- Tumour cells are uniform and *monotonous* in appearance, forming discrete islands, glands, cords or trabeculae.
- They have scanty cytoplasm and round-to-oval nucleus with fine stippled chromatin.
- Mitoses is infrequent and cellular atypia is uncommon.
- Other features: Presence of membrane-bound secretory granules (or dense core granules), containing chromogranin A, synaptophysin, neuron-specific enolase, etc.

Characteristic Features of Carcinoids in Specific Locations

Terminal Ileum

- Peak involvement in seventh decade
- Female predominance
- Multicentric
- Metastasize widely

Appendix

- Most common gut carcinoid
- Affects patients in the third and fourth decades of life
- Usually solitary
- Behave like locally malignant tumours/metastasis is rare (rectal and appendiceal carcinoids, almost never metastasize)

Hind Gut Carcinoids

- Constitute 10–20% of all cases
- Involve mainly rectum and colon

Foregut Carcinoids

- Argentaffin-negative
- **Seen in stomach, duodenum and oesophagus**

Carcinoid Syndrome

Salient Features

- It is present in 1% of all carcinoid tumour patients.
- More common in patients in whom the tumour has widely metastasized, particularly to the liver. Loss of liver function is essential for the syndrome to manifest, as liver normally converts active 5-HT (5-hydroxytryptamine or serotonin) into its inactive form 5-HIAA (5-hydroxy indole acetic acid).
- **Secretory product** mainly responsible for the syndrome is 5-HT; histamine, bradykinin, prostaglandins, etc., may also contribute.

Clinical Presentation

- Cutaneous flushes and cyanosis *(vasomotor disturbances)*
- Diarrhoea, cramps, nausea and vomiting *(due to intestinal hyper motility)*

- Cough, dyspnoea and wheezing *(asthmatic bronchoconstrictive attack due to released mediators)*
- Hepatic metastasis causing nodular liver *(hepatomegaly)* in some patients
- *Systemic fibrosis* involving heart (right-sided valvular stenosis and endocardial fibrosis), retroperitoneal and pelvic fibrosis in other patients

Q. Describe the clinicopathological features of acute appendicitis.

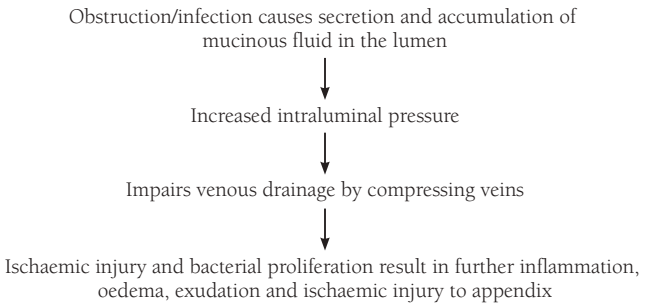
Ans. Acute appendicitis is defined as acute inflammation of appendix. It is generally seen in children and young adult and results from **obstruction,** which may be due to:

- Fecolith
- Tumour
- Foreign body
- Oxyuris vermicularis
- Diffuse lymphoid hyperplasia

Other Causes

- Inappropriate intake of roughage
- Haematogenous spread of infection to appendix
- Vascular occlusion
- Idiopathic

Pathogenesis (Flowchart 14.3)



FLOWCHART 14.3. Pathogenesis of acute appendicitis.

Morphology

The histological hallmark for the diagnosis of acute appendicitis is neutrophilic infiltration of the muscularis propria.

Normal Appendix

- Serosa is glistening and thin.
- No neutrophilic infiltrate observed in the muscle layer.

Acute Appendicitis

- Organ swollen with a hyperaemic, dull and granular mucosa
- Neutrophilic infiltration in mucosa, submucosa and muscularis propria
- Subserosal vessels congested

Acute Suppurative Appendicitis

- Serosa is coated with a fibrinopurulent exudate.
- Prominent ulceration and necrosis in the mucosa

15

Diseases of the Hepatobiliary System and Pancreas

LIVER

- Largest solid organ in the body (1200–1500 g)
- Divided into right and left lobes by the falciform ligament, the fissure of ligamentum teres and the fissure of ligamentum venosum
- Surgical division into **right and left hemilivers** by the middle hepatic vein, which lies between the inferior vena cava and the gallbladder, and passes through the porta hepatis
- The right and left hemilivers are further subdivided into a total of 8 segments in accordance with subdivisions of hepatic vasculature.
- Each segment is made up of histological units called **'lobules'**; each lobule is composed of a central vein, radiating sinusoids, separated from each other by plates of hepatocytes containing bile canaliculi and peripherally located portal tracts (Fig. 15.1). Hepatocytes are large polyhedral cells arranged as flat, anastomosing plates, one cell thick. Venous sinusoids have **kupffer cells** that are liver macrophages. Between the sinusoids and the hepatocytes are seen storage cells called **Ito cells**.
- The portal tracts contain branches of hepatic artery, portal vein, bile ducts and hepatic lymphatics, and comprise the main connective tissue stroma of the liver.
- Different regions of the lobule are referred to as **'periportal', 'mid-zonal' and 'centrilobular'.**
- Using the hepatic vasculature as reference, the liver architecture is divided into **'acini'.**
- On the basis of distance from the portal vessels, acinus is divided into **'zone 1' (closest to the portal vessels), 'zone 2' and 'zone 3' (farthest from the portal vessel).**
- Bile flows in the opposite direction along the **biliary canaliculi into terminal bile ductules (cholangioles)** and then **interlobular bile ducts** located in the portal tracts.

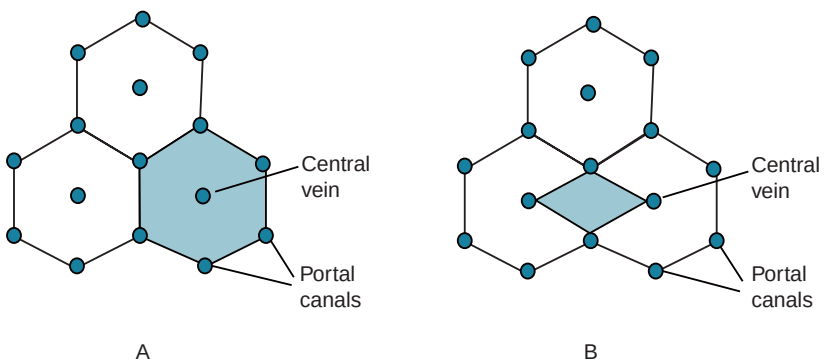


FIGURE 15.1. Schematic diagram of a (A) Lobule and (B) Acinus.

Q. Enumerate the important functions of liver.

Ans. Functions of Liver

1. **Metabolic**
   - Metabolism of carbohydrates, proteins and lipids
2. **Synthetic**
   - Albumin
   - Coagulation factors
   - Complement
   - Haptoglobin
   - Ceruloplasmin
   - Transferrin
   - Protease inhibitors
3. **Storage**
   - Iron
   - Copper
   - Vitamins A, D and B_{12}
4. **Excretion**
   - Bile salts
   - Bilirubin

Q. Enumerate and describe the tests to assess liver function.

Ans. Liver Function Tests

1. **Bilirubin in the blood (indicator of excretory function):** Bilirubin is derived from degradation of haemoglobin released from RBCs.
   (a) Normal serum bilirubin level is 0.3–1.0 mg/dL.
   (b) **Jaundice** occurs when bilirubin levels exceed 2 mg/dL of serum.
   (c) **Total bilirubin:** Bilirubin, which has not been metabolized
   (d) **Direct (conjugated) bilirubin:** Bilirubin, which has undergone conjugation and is water soluble.
2. **Bilirubin in the urine**
   (a) Normally, bilirubin cannot be detected in urine.
   (b) **Unconjugated hyperbilirubinaemia** is characterized by absence of bilirubin in the urine.
   (c) Since conjugated bilirubin is water soluble, bilirubinuria in a jaundiced patient points to **conjugated hyperbilirubinaemia (hepatobiliary disease).**
3. **Urine urobilinogen**
   (a) Urinary urobilinogen is detected by **Ehrlich's test.**
   (b) No urobilinogen is found in urine in obstructive jaundice.
   (d) Markedly increased urobilinogen is observed in urine in haemolytic disease.
4. **Liver enzymes:**
   The pattern of enzyme abnormalities changes with the type of liver injury as different hepatic enzymes are located in different locations within the hepatocyte. Lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are located in the cytoplasm. Mitochondrial isoenzyme of AST is specifically located in the mitochondria, and canalicular enzymes include alkaline phosphatase and γ-glutamyl transferase (GGT). The former are released in cytoplasmic and mitochondrial injury, respectively, and the latter in canalicular injury caused by obstructive processes. Different liver enzymes include
   (a) Aminotransferases (indicator of liver cell necrosis)
      (i) There are two enzymes in this category, AST, also known as serum glutamate oxaloacetate transaminase (SGOT) and ALT, formerly called serum glutamate pyruvate transaminase (SGPT).
      (ii) ALT is more specific for hepatocellular damage because the activity of ALT outside the liver is low and it is found primarily in the liver. Normal value is 0–45 IU/L.

(iii) AST on the other hand is also found in heart and muscle. Normal value is 0–40 IU/L.
(iv) Aminotransferase levels are useful in differentiating between hepatocellular and obstructive jaundice.
(v) Marked elevation is seen in severe viral hepatitis (Hepatitis A, B and C), drug-induced injury (acetominaphen toxicity) and circulatory abnormalities (shock liver).
(vi) Mild elevation is seen in neonatal hepatitis, extrahepatic biliary atresia, fatty liver, cirrhosis, NASH (nonalcoholic steatohepatitis) and drug toxicity.

(b) Alkaline phosphatase or ALP (indicator of cholestasis)
(i) Serum ALP activity is primarily derived from liver and bone. Normal level is 3–13 KA units.
(ii) In **hepatocellular jaundice,** very little ALP is liberated from the cells and the rise in ALP is less than three folds.
(iii) In **obstructive jaundice,** due to obstruction of biliary tract, all the new ALP that is synthesized, escapes into the blood. Hence, serum ALP levels are markedly raised.

***Causes of raised ALP*:**
- Obstructive jaundice
- Metastatic bone tumours
- Hyperparathyroidism
- Paget disease
- Pregnancy
- Rickets
- Tumours of GIT

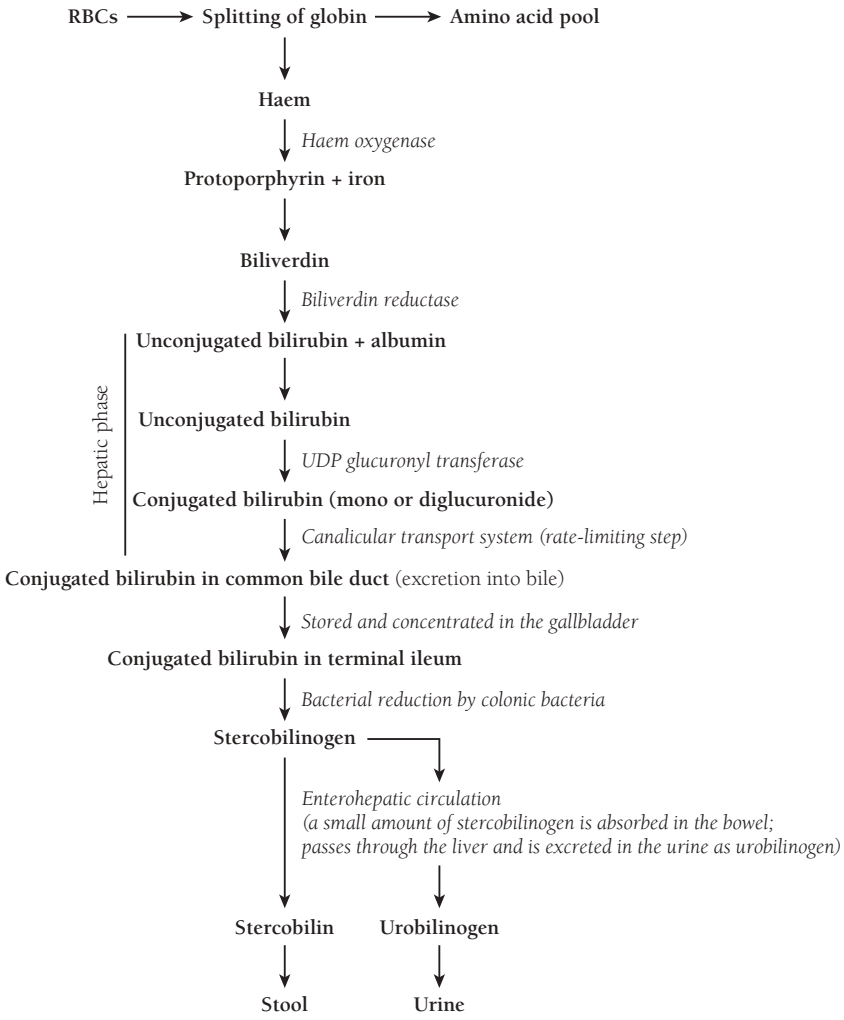
(c) Gamma-glutamyl transpeptidase or GGTP (indicator of cholestasis)
(i) If the source of ALP is not clear, the levels of two enzymes, **gamma-glutamyltransferase** and **5' nucleotidase** can be determined (more specific for liver). Raised levels occur in biliary obstruction and parenchymal damage.
(ii) Serum levels rise in acute and chronic alcoholism (raised levels suggest prolonged intake of more than 60 g alcohol/day).

5. **Plasma proteins (indicator of synthetic ability):**
(a) **Albumin** is synthesized in liver. Normal serum albumin level is 3.5–4.5 g/dL. In chronic liver diseases like cirrhosis and chronic active hepatitis, serum albumin is low (<3 g/dL).
(b) **Globulins** are synthesized by the reticuloendothelial system. Normal serum globulin level is 1.5–3 g/dL. Their levels rise in chronic liver disease. IgG is raised in chronic active hepatitis and cryptogenic cirrhosis. IgA is raised in alcoholic liver disease. IgM is raised in primary biliary cirrhosis.
6. **Coagulation factors (indicator of synthetic ability):**
(a) Liver synthesizes 11 coagulation factors and activates some in the presence of vitamin K.
(b) Prothrombin time (PT) is prolonged in liver disease (PT depends on factors I, II, V, VII and X, and gets prolonged when the plasma concentration of any one of these falls below 30% of the normal).
7. **Bromsulphthalein (BSP) clearance:** BSP clearance is delayed in Dubin–Johnson syndrome.
8. **Other tests for liver function:**
(a) Serology for viral hepatitis (B and C, CMV and EBV)
(b) Autoantibody screen for autoimmune hepatitis and biliary cirrhosis (antimitochondrial antibody, antismooth muscle antibody and antinuclear antibody)
(c) Serum ferritin and transferrin saturation for haemachromatosis
(d) α-fetoprotein levels for hepatocellular carcinoma
(e) Copper/ceruloplasmin levels for Wilson disease
(f) α-1 antitrypsin levels for α-1 antitrypsin deficiency
(g) Noninvasive tests like ultrasound and CT that help in detecting structural abnormalities
(h) Doppler test can be used to assess vasculature-related abnormalities
(i) Liver biopsy for definitive histopathological diagnosis of inflammatory and neoplastic pathology

Q. Write briefly on bilirubin metabolism.

Ans. Metabolism of Bilirubin (Flowchart 15.1):

- **80–85%** of bilirubin is derived from the catabolism of the haemoglobin of senescent red blood cells.
- **15–20%** is derived from the bone marrow, destruction of maturing cells, liver and the turnover of haem and haem-containing precursors (cytochromes, myoglobin, etc.).



FLOWCHART 15.1. Bilirubin metabolism.

Q. Define and classify jaundice.

Ans. Bilirubin and cholesterol have low water solubility and cannot be excreted into urine. Bile is the primary pathway for elimination of both. Hepatocellular damage leads to a disruption in bile metabolism and manifests clinically as *jaundice* (yellowish pigmentation of skin and mucous membranes) and *icterus* (yellow discoloration of sclera). The latter occurs because bilirubin has a special affinity for elastin which is present abundantly in the sclera. Yellow discoloration is also prominent in the palpebral conjunctiva, sublingual mucosa and lower abdominal skin.

Clinical Features of Jaundice

- Jaundice (excess bilirubin deposited in the skin and mucosae)
- Dark urine (results from excess bilirubin excreted by the kidneys)
- Light-coloured stools (passage of bilirubin into the intestine is blocked)
- Generalized itchiness (retention of bile products in the skin may cause itching, with subsequent scratching and skin damage)
- Stools may contain too much fat (a condition called steatorrhoea) because bile cannot enter the intestine to help digest fat in foods
- There is impaired absorption of calcium, vitamin D and K due to decreased entry of bile in the intestine. The patient has a tendency to bleed due to deficiency of vitamin K.

Classification of Jaundice

The classification of jaundice is based on the **pathological mechanisms** underlying it (Table 15.1):

1. **Haemolytic jaundice**
   (a) Increased destruction of red blood cells or their precursors, resulting in a predominant increase in unconjugated bilirubin
   (b) Absence of bilirubin in urine
   (c) Urinary urobilinogen is increased (more than 4 mg/24 h).
   (d) Other liver function tests are normal.
   (e) Evidence of haemolytic anaemia (increased reticulocyte count, or presence of fragmented red cells or Schistocytes in the peripheral blood film, decreased haptoglobin, increased LDH and positive direct Coombs test)
2. **Hepatocellular jaundice:** In hepatocellular jaundice, concentration of both unconjugated and conjugated bilirubin is increased. It has two elements, an 'obstructive element', causing impaired uptake of unconjugated bilirubin into the cell and of conjugated bilirubin into biliary canaliculi. Swelling of cells and oedema due to inflammation contribute to mechanical obstruction of the intrahepatic biliary tree. The 'hepatocellular element' results from the liver cell damage.
   ***Indicators of hepatocellular injury*:**
   - Elevated aminotransferase activity
   - Acute phase reactant response (iron and ferritin elevation)
   - Reduced synthetic function (prolonged PT, low albumin and cholesterol)

TABLE 15.1. Pathophysiological classification of jaundice

Type	Mechanism	Causes
Prehepatic	Increased production of bilirubin	Haemolysis (intravascular or extravascular) Ineffective erythropoiesis Haemorrhagic infarction Massive hematomas
Hepatic	Reduced uptake	**Congenital**: Gilbert syndrome **Acquired**: Drugs (rifampin and contrast dyes), septicaemia, fasting
	Impaired conjugation	Physiological jaundice of newborn **Congenital**: Gilbert, Crigler–Najar syndromes **Acquired**: Hepatitis, benign and malignant neoplasms
	Reduced excretion into the bile	**Congenital**: Dubin–Johnson and Rotor syndromes **Acquired**: Drugs (oral contraceptives, methyl testosterone, chlorpromazine), hepatitis, biliary cirrhosis and benign cholestasis of pregnancy
Posthepatic	Obstruction of bile ducts	Stones, pancreatitis, pancreatic tumour, parasites, strictures, tumours and biliary atresia (intrahepatic and extrahepatic)

3. **Cholestatic (surgical) jaundice**
   (a) Cholestasis means failure of the bile flow and its cause may lie anywhere between the hepatocyte and duodenum.
   (b) Cholestasis can be due to small duct obstruction (intrahepatic cholestasis) or large duct obstruction (extrahepatic cholestasis). Large bile duct obstruction is mainly due to gallstones and malignancies of the head and neck of pancreas.
   Indicators of cholestasis
   - Hyperbilirubinaemia and bilirubinuria
   - Elevated alkaline phosphatase activity
   - Elevated **gamma glutamyl transferase, 5′ nucleotidase** and **leucine amino peptidase**
   - Hypercholesterolaemia
   - High serum bile salts (mainly cholate and chenodeoxycholate)

The laboratory tests to differentiate different types of jaundice are enumerated in Table 15.2.

TABLE 15.2. Laboratory tests to differentiate between different types of jaundice

Features	Prehepatic jaundice	Hepatic jaundice	Posthepatic jaundice
Serum			
Total bilirubin	Normal/increased	Increased	Increased
Conjugated bilirubin	Normal	Normal/decreased	Increased
Unconjugated bilirubin	Increased	Normal/increased	Normal
Urobilinogen	Increased	Normal/Increased	Decreased/negative
Urine			
Bilirubin in urine	Absent	Absent	Present
Urinary urobilinogen	Increased (more than 4 mg/24 h)		
Peripheral smear	Evidence of haemolysis (increased reticulocyte count, schistocytes or fragmented red cells in the peripheral blood film, decreased haptoglobin, increased LDH and positive direct Coombs test)	Not seen	Not seen

Q. Write briefly on congenital nonhaemolytic hyperbilirubinaemias.

Ans. Congenital nonhaemolytic hyperbilirubinaemias include

Gilbert Syndrome

- Autosomal recessive inheritance
- Mild deficiency of UGT1A1 (Uridine diphosphate–glucuronyltransferase); Levels are reduced to 10–35% of normal and result in unconjugated hyperbilirubinaemia

Crigler–Najjar Syndrome—Type I

- Autosomal recessive inheritance
- Complete absence of UGT1A1 activity
- Severe unconjugated hyperbilirubinaemia and kernicterus leading to neonatal death

Crigler–Najjar Syndrome—Type II

- Autosomal dominant inheritance
- Partial deficiency of UGT1A1
- Jaundice is milder than type I, kernicterus is occasionally seen

Dubin–Johnson Syndrome

- Autosomal recessive inheritance
- Decreased canalicular excretion of bilirubin into biliary canaliculi
- Conjugated hyperbilirubinaemia and bilirubinuria
- The degree of hyperbilirubinaemia may be increased by intercurrent illness, oral contraceptives and pregnancy.
- The syndrome is due to defective **MRP2** (multidrug resistance-associated protein 2), which is required for secretion of conjugated bilirubin from the hepatocytes into canaliculi.
- **BSP (bromsulphthalein) retention test shows impaired clearance with a reflux back into blood in 90 min.**
- Hepatomegaly with a **dark pigment** in centrilobular hepatocytes (derived from polymerized epinephrine metabolites)

Rotor Syndrome

- Autosomal recessive inheritance
- Due to poor uptake and storage of bilirubin by liver cells
- Mild jaundice, conjugated hyperbilirubinaemia and bilirubinuria
- BSP retention test shows impaired clearance but there is no reflux back into blood.
- Liver biopsy is normal and does not show dark pigment.

Q. Outline the aetiology, epidemiology, clinical features and laboratory diagnosis of viral hepatitis.

Ans. ***Types of Viral Hepatitis***

1. Hepatitis A caused by hepatitis A virus (HAV)
2. Hepatitis B caused by hepatitis B virus (HBV)
3. Delta hepatitis caused by hepatitis D virus (HDV)
4. Hepatitis C caused by hepatitis C virus (HCV)
5. Hepatitis E caused by hepatitis E virus (HEV)
6. Hepatitis G virus
7. Hepatitis caused by other viruses (cytomegalovirus, Epstein–Barr virus, Herpes simplex virus and yellow fever virus)

1. **Hepatitis A**
   Aetiology
   Caused by hepatitis A virus (HAV), an RNA virus belonging to the Picornavirus group
   Epidemiology
   - Incubation period is 2–6 weeks
   - HAV is transmitted almost exclusively by the feco-oral route (infected persons excrete the viruses in their faeces for two weeks before the onset and one week after the onset of the illness). Source of the infection is contaminated water, milk and raw or steamed shell fish.
   - Can rarely spread by blood transfusions and homosexual activity
   - Does not cause chronic liver disease or carrier state; rarely causes fulminant hepatitis (0.1% case fatality)
   - It is more common in children and rare in adults.
2. **Hepatitis B**
   Aetiology
   - Caused by an enveloped DNA virus called hepatitis B virus (HBV), belonging to the group of **hepadnaviruses**
   - HBV is a 42 nm **'Dane particle'** composed of
     - A **surface envelope** (antigen expressed on it is called hepatitis B surface antigen, **HBsAg**)
     - A **nucleocapsid core** containing DNA (antigen expressed on its surface is called hepatitis B core antigen or **HBcAg**)

- Another soluble antigen in the nucleocapsid is called hepatitis B e-antigen or **HBeAg**)
- A **DNA polymerase enzyme (pol)** that exhibits reverse transcriptase activity
- A protein from the X region, **HBX** necessary for viral replication; HBX controls gene transcription and thus acts as a gatekeeper for hepatocyte check points in cell cycle.

- **The corresponding antibodies are**
  - Anti-HBs
  - Anti-HBc
  - Anti-HBe

***Serological diagnosis* (*Fig. 15.2*):**

- Serum HBsAg is the first serum virological marker to appear. It appears in the later part of the incubation period or in the early prodrome of hepatitis B. Peak levels are reached during acute disease and the levels decline to undetectable levels in 3–6 months. Antibody to HBsAg is detected in the serum after HBsAg disappears.
- HBeAg, HBV–DNA and DNA polymerase are detected in the serum immediately after the appearance of HBsAg. Their presence indicates active viral replication.
- IgM anti-HBc appears in serum just before the patient manifests with acute disease (it is the earliest antibody to appear and is replaced by IgG anti-HBc over a period of few months.
- Persistence of detectable HBsAg beyond 6 months suggests chronic hepatitis B infection. In such cases, anti-HBs becomes negative but anti-HBc remains detectable.
- **Indicators of chronic replication:**
  - Persistence of circulating HBsAg, HBeAg and HBV DNA
  - Presence of anti-HBc and occasionally with anti-HBs

Epidemiology

- Incubation period is about 1–4 months.
- Asymptomatic carriers or persons with acute hepatitis or chronic liver disease are the source of infection. It is present in all body fluids except stool.
- The main route of transmission of Hepatitis B is parenteral (commonly occurs after transfusion of infected blood or blood products, injections with contaminated needles, dialysis, tattooing and acupuncture). It spreads through body fluids like saliva, urine, semen and vaginal secretions.
- Mother-to-child spread (vertical or perinatal transmission) is also common.
- High-risk groups include spouses of persons having acute infection, homosexuals, healthcare workers, dentists and haemophiliacs.
- The incidence of a chronic carrier state in HBV infection varies between 1% and 20%.

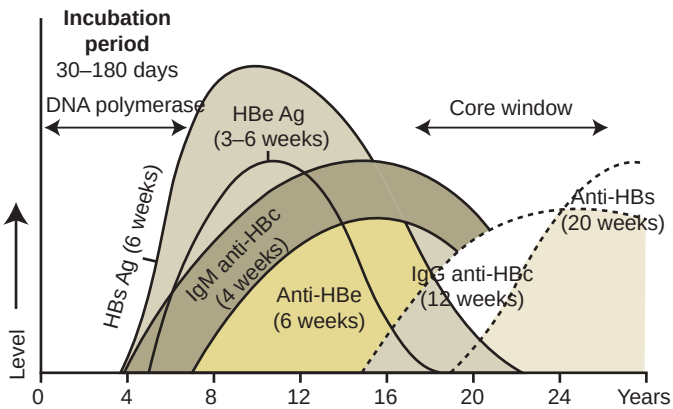


FIGURE 15.2. Serological diagnosis of hepatitis B.

3. **Delta hepatitis (hepatitis D)**
   Aetiology
   - It is caused by hepatitis D virus (HDV), which is a defective RNA virus. The RNA genome is encapsulated or covered by an outer coat of hepatitis B surface antigen.
   - It requires hepatitis B virus for replication and expression.
   - Incubation period is 1–4 months.
   - HDV RNA is detectable in the blood and liver just before and during acute symptomatic disease.
   - HDV can infect a person simultaneously with HBV **(coinfection)** or it may super infect a person who is already a chronic carrier of HBV **(super infection).**
   - Acute coinfection by HBV and HDV is best suggested by presence of IgM against both HDAg and HBcAg.

   Epidemiology

   Two epidemiological patterns exist:
   - Predominant transmission by nonparenteral route, especially close personal contact **(endemic areas)**
   - Predominant transmission by parenteral route, ie, persons exposed frequently to blood and blood products, mainly intravenous drug addicts and haemophiliacs **(nonendemic).**
4. **Hepatitis C**
   Aetiology
   - Formerly called blood-borne, non-A and non-B hepatitis, it is a single-stranded RNA virus belonging to the family *Flaviviridae*. It shows a lot of genomic instability and antigenic variability, thereby making it difficult to develop a vaccine against it.
   - HCV RNA can be detected in the course of infection, well before the appearance of antibodies to HCV.

   Epidemiology
   - Incubation period is 6–8 weeks.
   - HCV is the cause of greater than 90% cases of post-transfusion hepatitis. Perinatal and sexual transmission can occasionally be seen.
   - Carrier state is quite common with hepatitis C infection.
   - Anti-HCV antibody is found to be positive in more than 50% cases of unexplained cirrhosis or hepatocellular carcinoma.
5. **Hepatitis E**
   Aetiology
   - It is a nonenveloped single-stranded RNA virus belonging to the Hepevirus genus.
   - It is responsible for 40–60% cases of acute hepatitis in India. HEV antigen can be identified in the cytoplasm of hepatocytes during active infection. The virus itself can be isolated from the stools of the patient and anti-HEV IgG and IgM antibodies can be detected in serum.

   Epidemiology
   - Incubation period is 4–5 weeks.
   - Primary mode of transmission is enteric (epidemic, water-borne hepatitis). Source of infection is animal reservoirs (monkeys, dogs, pigs and cats).
   - A characteristic feature of HEV infection is the high mortality rate among pregnant women.
6. **Hepatitis G**
   - HGV is similar to viruses in the *Flaviviridae* family.
   - Can be transmitted by blood transfusion.
   - HGV coinfection is observed in 6% of chronic HBV infections and in 10% of chronic HCV infections; however, *whether HGV is actually pathogenic in humans remains unclear*.

Clinically viral hepatitis evolves through the following stages: (*i*) asymptomatic, (*ii*) acute and (*iii*) chronic.

Asymptomatic Phase

Patients are identified incidentally based on elevated aminotransferases or presence of serological markers.

Acute Viral Hepatitis

Acute viral hepatitis is further subdivided into the following clinical stages:

1. *Incubation period.*
2. *Symptomatic preicteric phase*: Also called prodromal phase, it lasts for a few days up to 2 weeks before the onset of jaundice, and manifests with fever, headache, malaise, anorexia, nausea, vomiting, diarrhoea, distaste for cigarettes and upper abdominal pain (due to stretching of liver capsule). Patients with HBV infection occasionally have a 'serum sickness-like syndrome' with skin rashes and polyarthralgia.
3. *Symptomatic icteric phase*: This is characterized by conjugated hyperbilirubinaemia with passage of dark urine and yellowish discolouration of the sclera. The constitutional symptoms diminish with the onset of clinical jaundice when the patient develops tender hepatomegaly. With progressively increasing obstruction to biliary canaliculi, jaundice worsens, stools become paler, urine becomes darker and liver becomes more palpable (cholestatic phase). Icteric phase is seen in HAV infection, but is rare in HBV and HCV infections.
4. *Convalescence or recovery phase*: There is improvement in the gastrointestinal symptoms; decrease in jaundice, normalization of stools and urine and decrease in the liver size. The clinical and biochemical recovery should be complete in 1–2 months from the onset in cases of hepatitis A and E and in 3–4 months from the onset in hepatitis B and C.

Points to Remember

Delta coinfection is indistinguishable from acute hepatitis B, but **delta super infection** appears like an acute episode in a person chronically infected with HBV.

Hepatitis B, D and E can result in fulminant hepatic failure. It is uncommon with hepatitis A and C. Pregnant women suffering from hepatitis E have a high incidence of fulminant hepatitis (20%).

Anicteric hepatitis is a mild illness with an anicteric course (no clinical jaundice).

Morphological Features (Fig. 15.3)

- **Hepatocyte injury** and **ballooning degeneration** (swelling of hepatocytes with empty looking cytoplasm due to clumping of the cytoplasm around the nucleus)
- **Cholestasis** (seen as canalicular bile plugs)

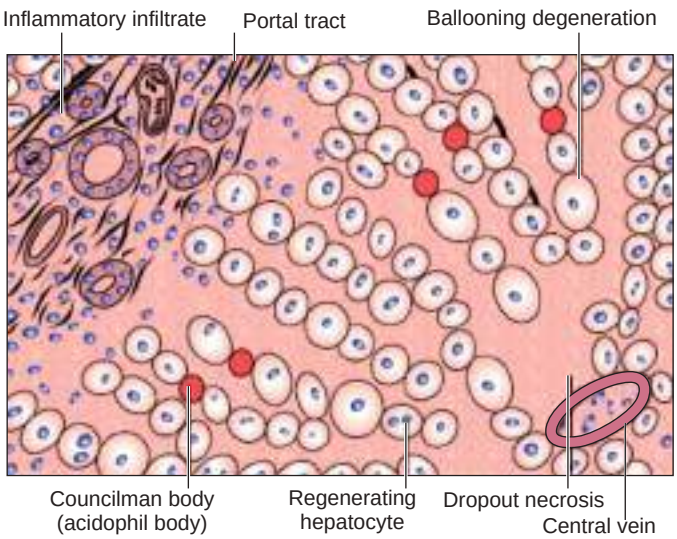


FIGURE 15.3. Section from acute viral hepatitis showing hepatocyte necrosis and periportal inflammation.

- **Hepatocyte necrosis** (necrosis of isolated cells or clusters) seen as cytolysis (dropout necrosis) and apoptosis (formation of apoptotic or Councilman bodies)
- **Bridging necrosis** (confluent necrosis of hepatocytes connecting portal–portal, portal–central, central–central areas).
- **Lobular disarray** leading to loss of normal architecture
- **Regenerative changes** including hepatocyte proliferation and reactive sinusoidal changes (Kupffer cell hyperplasia and hypertrophy)
- **Portal tracts** show periportal inflammation (mainly mononuclear) with inflammatory spillover into adjacent parenchyma and hepatocyte necrosis.
- **HCV** infection is commonly associated with duct proliferation, lymphoid aggregates in the portal tracts and mild fatty change.
- **HBV**-induced changes include development of fine granularity in the cytoplasm of liver cells or **ground glass appearance** due to the accumulation of spheres and tubules of HBsAg and **sanded nuclei** due to abundant intranuclear HBcAg.

Complications

- Fulminant hepatic failure
- Chronic hepatitis
- Cirrhosis
- Hepatocellular carcinoma
- Hepatocellular failure
- Renal failure

Chronic Hepatitis

It is defined as symptomatic, biochemical or serological evidence of continuing or relapsing hepatic disease for more than 6 months with histologically documented inflammation and necrosis.

Causes

- Chronic viral hepatitis
- Wilson disease
- α-1 antitrypsin deficiency
- Chronic alcoholism
- Drugs—isoniazid, methyl dopa and methotrexate
- Autoimmune hepatitis
- Cryptogenic chronic hepatitis

Clinical Features

- Persistent elevation of serum aminotransferases
- Fatigue, malaise, loss of appetite and mild jaundice
- Spider angiomas, palmar erythema, mild hepatomegaly and hepatic tenderness
- Prolonged prothrombin time, hypergammaglobulinaemia, hyperbilirubinaemia and mild increase in alkaline phosphatase
- In HBV and HCV disease, circulating immune complexes may cause vasculitis and glomerulonephritis

Classification

Old classification of chronic hepatitis

1. **Chronic persistent hepatitis (CPH)**
   (a) Infiltration by chronic inflammatory cells is confined to the portal tracts.
   (b) Changes in hepatocytes are absent or slight (‘**spotty necrosis**’ or small foci of liver cell necrosis with inflammatory cell infiltration).
   (c) Lobular architecture is maintained.
   (d) Prognosis is excellent. Rarely, may progress to chronic active hepatitis or cirrhosis.

2. **Chronic lobular hepatitis (CLH)**
   (a) Uncommon disease with evidence of hepatitis B or hepatitis C virus infection.
   (b) Antinuclear antibodies, antismooth muscle antibodies and antimitochondrial antibodies in some patients.
   (c) Live biopsy shows features similar to acute viral hepatitis.
3. **Chronic active hepatitis (CAH)**
   (a) Both portal tracts and parenchyma are involved.
   (b) Lobular architecture is distorted.
   (c) Portal tract inflammation spills over into surrounding parenchyma.
   (d) **'Piecemeal necrosis'** and **'bridging hepatic necrosis'** seen.
   (e) Regenerative nodules develop and later progress to cirrhosis.

New classification of chronic hepatitis is based on:

1. Cause of hepatitis
2. Histological activity or grade
3. Degree of progression or stage

Grading of chronic hepatitis is based on the histopathological evidence of inflammation and necrosis. Proportionate to the severity of following factors, a severity score (mild, moderate or severe) is as signed.

1. *Periportal necrosis including piecemeal necrosis and/or bridging necrosis*
2. *Intralobular necrosis*
3. *Portal inflammation*
4. *Fibrosis*

Staging of chronic hepatitis is based on the degree of fibrosis (stage 0 with no fibrosis to stage 4 with cirrhosis).

Morphological Features (Fig. 15.4)

- Hepatocyte injury and regeneration.
- Sinusoidal cells show reactive changes.
- Portal inflammation with or without spillover in the adjacent parenchyma is seen.
- Spillover of inflammation in the adjacent parenchyma causes necrosis of adjacent hepatocytes **(interface hepatitis).**
- Fibrosis **(portal, periportal and bridging)** may follow.

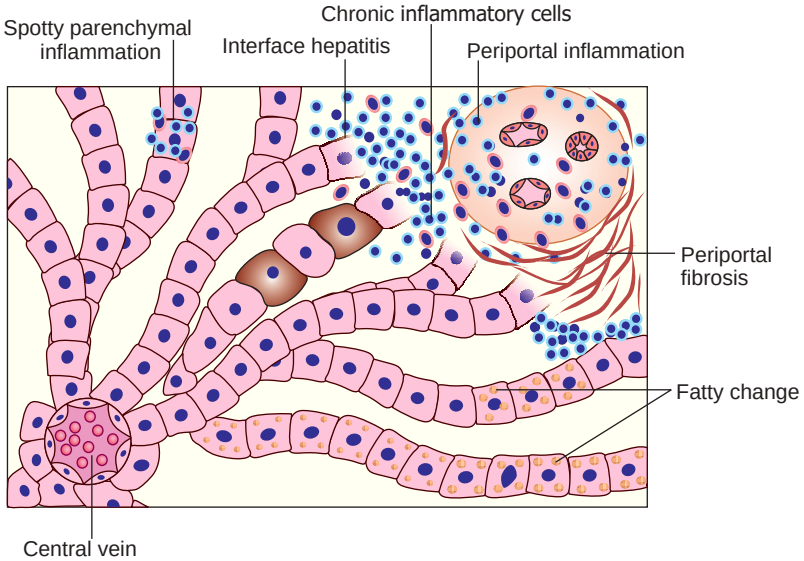


FIGURE 15.4. Section from chronic hepatitis showing portal inflammation with spillover in the adjacent parenchyma.

Salient features of different types of hepatitis have been given in Table 15.3.

TABLE 15.3. Comparative features of different types of hepatitis

Features	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E	Hepatitis G
Agent	Icosahedral capsid, ssRNA	Enveloped dsDNA	Enveloped ssRNA	Enveloped ssRNA	Unenveloped ssRNA	ssRNA
Incubation period	2–6 weeks	4–26 weeks	2–26 weeks	4–7 weeks	2–8 weeks	Unknown
Transmission	Feco-oral	Parenteral, close contact	Parenteral, close contact	Parenteral, close contact	Waterborne	Parenteral
Carrier state	None	Present	Present	Present		Present
Chronic hepatitis	None	5–10% of acute infections	>50%	<5% coinfection, 80% super infection	None	None
Hepatocellular carcinoma	No	Yes	Yes	No increase above HBV	Unknown	None
Diagnosis	Detection of serum IgM antibodies	Detection of HBsAg or antibody to HBcAg	PCR for HCV RNA; third-generation ELISA for antibody detection	Detection of IgM and IgG antibodies; HDV RNA in serum and HDAg in liver	PCR for HEV RNA; Detection of serum IgM and IgG antibodies	Not a primary hepatotropic virus; replicates in the bone marrow and spleen

Q. Define fulminant hepatic failure and write briefly on its causes and clinicopathological features.

Ans. Fulminant hepatic failure is defined as sudden loss of hepatic function, occurring within 4 weeks of onset of the precipitating illness, in the absence of any evidence of pre-existing liver disease. More protracted course over months is labelled submassive or subacute hepatic necrosis.

Aetiology

- Acute viral hepatitis (A, B and E)
- Hepatotoxic drugs (isoniazid and phenytoin)
- Poisoning, eg, *Amanita phalloides*
- Shock
- Wilson disease

Pathology

- Shrinkage of liver with extensive parenchymal necrosis
- Complete destruction of hepatocytic lobules leaving only preserved portal tracts
- Collapse of reticulin framework
- Survival beyond day's influx of inflammatory cells; survival more than a week regeneration of surviving hepatocytes seen

Clinical Features

- Weakness, nausea, vomiting, right hypochondrial pain and jaundice
- Features of hepatic encephalopathy and cerebral oedema
- Renal failure

Q. Write briefly on Reye syndrome.

Ans. Reye syndrome is mainly seen in children and adolescents, and is rare in adults.

- It is characterized by severe fatty degeneration of the liver and usually follows a viral illness.
- History of aspirin intake may be elicited.
- It manifests with acute encephalopathy with cerebral oedema and is a prototype of conditions called **'mitochondrial hepatopathies'** (causes generalized loss of mitochondrial function).

Q. Write briefly on autoimmune (lupoid) hepatitis.

Ans. It is a chronic hepatitis with multiple immunologic abnormalities. The following are the salient features of autoimmune hepatitis:

- Female preponderance; HLA association (association with HLADRB1 allelles in Caucasians)
- Insidious onset with fatigue, anorexia and jaundice
- Signs of chronic liver disease (spider telangiectasia and hepatosplenomegaly)
- Coexistence of other autoimmune diseases (rheumatoid arthritis, thyrotoxicosis, Hashimoto thyroiditis, myxoedema, Coombs positive haemolytic anaemia)
- Absence of serologic markers of viral infections
- Elevation of serum IgG (>2.5 g/dL) and high titres of autoantibodies eg, antinuclear antibodies (ANAs), antismooth muscle antibodies (SMAs) like antibodies to actin, troponin and tropomyosin, antisoluble liver antigen/liver pancreas antigen (SLA/LP) antibodies, antiliver cytosol 1 (ACL-1) antibodies. liver–kidney microsomal antibody directed against cytochrome P450 and antisoluble liver/kidney microsomes (anti-LKM1 antibody).
- Two types of autoimmune hepatitis are identified – Type 1 is more common in older individuals and shows positivity for ANA, SMA, anti-SLA/LP and AMA and Type 2 which affects children and young adults and shows positivity for anti-LKM-1 antibodies and ACL-1 antibodies.
- Confluent necrosis, severe interface hepatitis, predominance of plasma cells and rosetting of hepatocytes are diagnostic histopathological features.

Q. Define cirrhosis. Enumerate its causes and consequences.

Ans. Cirrhosis is a diffuse liver disease characterized by:

1. Widespread hepatocyte necrosis with simultaneous regeneration leading to the formation of nodules of various sizes (**micronodules** less than 3 mm and **macronodules** more than 3 mm; Figs. 15.5A and B).
2. Bridging fibrous septae, which distort the hepatic architecture.
3. Destruction and distortion of hepatic vasculature by fibrosis, which eventually leads to the formation of **portosystemic shunts (portal hypertension and its sequelae; eg, gastroesophageal varices)** and **splenomegaly.**

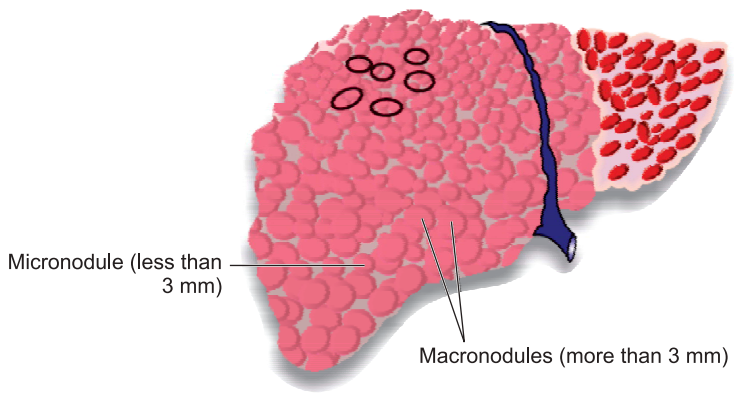


FIGURE 15.5A. Schematic diagram of cirrhosis of liver showing micro- and macronodules.

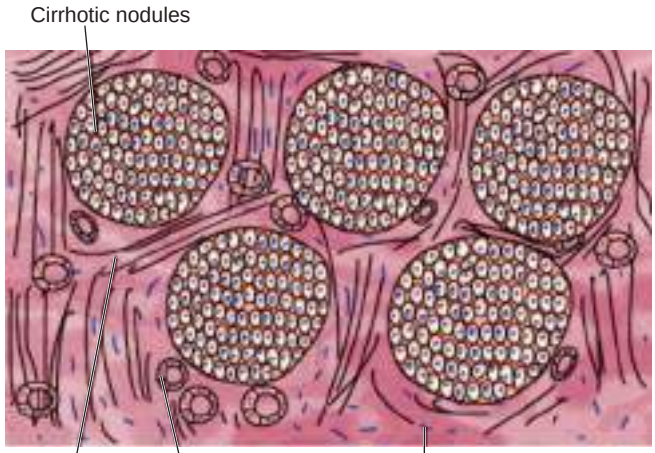


FIGURE 15.5B. Portal cirrhosis liver.

4. **Ascites** and **hepatic encephalopathy** resulting from both hepatocellular insufficiency and portal hypertension. Hepatocellular damage also leads to **jaundice, oedema, coagulopathies** and **a variety of metabolic abnormalities.**

Classification

1. ***Aetiological***
   - Alcoholic cirrhosis
   - Nonalcoholic steatohepatitis (NASH)
   - Postnecrotic cirrhosis
   - Biliary cirrhosis (primary and secondary)
   - Hepatitis B, C and Delta
   - Haemochromatosis
   - Wilson disease
   - α-1 antitrypsin deficiency
   - Chronic autoimmune hepatitis
   - Drug-induced cirrhosis (methyldopa, isoniazid and methotrexate)
   - Inborn errors of metabolism (glycogen storage diseases and galactosaemia)
   - Cardiac cirrhosis
   - Cryptogenic cirrhosis
2. ***Morphological***
   - Micronodular cirrhosis
   - Macronodular cirrhosis
   - Mixed cirrhosis

Pathogenesis

- In the **normal liver**, ECM consists of collagen Types I, III, V and XI present around central veins, in portal tracts and in the liver capsule.
- Liver does not have a true basement membrane; instead, Type IV collagen and other proteins present in the space of Disse (space between sinusoidal endothelial cells and hepatocytes) form the supporting framework.
- Collagen is synthesized by Ito cells (perisinusoidal stellate or fat-storing cells), which lie in the space of Disse. These cells normally function as storage cells for vitamin A and fat, and become activated to myofibroblast-like cells under stimulation by reactive oxygen species (ROS), growth factors and cytokines like TNF, IL-1 and lymphotoxins.
- In cirrhosis, Types I and III collagen and other ECM components are deposited in the space of Disse. This leads to loss of sinusoidal endothelial cell fenestrations, which hamper the free exchange of solutes between plasma and hepatocytes.

- Movement of proteins (albumin, clotting factors and lipoproteins) between plasma and hepatocytes is markedly impaired, leading to functional changes in the liver.

Clinical Features

- Low-grade fever, weakness, fatigue, weight loss, anorexia, nausea, vomiting, upper abdominal discomfort and abdominal distension due to ascites
- Menstrual irregularities like amenorrhea and irregular menses, hypogonadism, diminished body hair and gynaecomastia (due to impaired oestrogen metabolism and resulting hyperestrogenaemia)
- Haemorrhagic tendencies like easy bruising, purpura, epistaxis, menorrhagia and gastrointestinal bleeding (decreased production of coagulation factors by the liver and thrombocytopenia resulting from hypersplenism)
- Portal hypertension and its sequelae

Signs of Hepatocellular Failure

- Jaundice (due to abnormal bilirubin metabolism)
- Palmar erythema and spider naevi (due to localized vasodilatation)
- Parotid enlargement (attributed to fatty infiltration since liver's ability to break down body fat is reduced in cirrhosis)
- Ascites (due to portal hypertension; and low levels of albumin in the blood)
- Hepatic encephalopathy and flapping tremors (associated with increased blood ammonia levels)
- Progressive renal dysfunction (due to decreased renal perfusion attributed to systemic vasodilatation)

Q. Outline the aetiopathogenesis and clinical features of portal hypertension.

Ans. Portal hypertension is defined as a clinical condition in which there is prolonged elevation of portal venous pressure due to increased resistance to portal blood flow.

Causes

- **Prehepatic:** Portal vein thrombosis and fibrosis of bile ducts (schistosomiasis)
- **Intrahepatic:** Cirrhosis, schistosomiasis, massive fatty change, sarcoidosis and miliary tuberculosis
- **Posthepatic**: Obstruction of hepatic vein by thrombosis (Budd–Chiari syndrome) or tumours

Pathogenesis of Portal Hypertension in Cirrhosis (Flowchart 15.2)

Perivenular fibrosis and compression of sinusoids by parenchymal nodules

↓

Increased resistance to blood flow at the level of sinusoids

↓

Increased portal vascular resistance leads to:
- Reduction in the flow of portal blood to the liver
- Development of collateral vessels allowing portal blood to bypass the liver and enter systemic circulation

↓

- Collateral vessel formation occurs in the oesophagus, stomach, rectum, anterior abdominal wall and in the renal, lumbar, ovarian and testicular (spermatic) vasculature
- With the development of collateral vessels, initially some of the portal blood and later almost all of the portal blood is shunted directly to the systemic circulation, bypassing the liver

FLOWCHART 15.2. Pathogenesis of portal hypertension in cirrhosis.

Clinical Features

- **Haematemesis** and **melena** (from variceal bleeding)
- **Fetor hepaticus** (a musty odour of breath, resulting from portal-systemic shunting of blood, which allows mercaptans to pass directly to the lungs. Mercaptans are formed by the action of GIT bacteria on methionine)
- **Caput medusae** (a number of prominent collateral vessels radiating from the umbilicus)
- **Splenomegaly** (an important diagnostic sign of portal hypertension)
- **Hypersplenism** (manifests as thrombocytopenia and leucopenia)
- **Small, contracted** and **fibrotic liver** (usually seen associated with very high portal venous pressure)
- **Haemorrhoids** (which occur from dilatation of rectal veins)
- **Ascites** (which occurs due to portal hypertension and hypoalbuminaemia due to liver cell failure)

Q. Outline the salient features of hepatorenal failure.

Ans. Hepatorenal syndrome is defined as renal failure associated with chronic liver disease. Kidneys are histologically normal and their function reverts to normal after reversal of hepatic failure. Renal failure is thought to result from **diminished renal blood flow**. In cirrhosis, circulatory changes lead to increased peripheral blood flow and decreased visceral blood flow, especially to the kidneys (this clinically manifests as a drop in urine output and rising blood urea and creatinine levels).

Q. Write briefly on alcoholic liver disease.

Ans. The spectrum of alcoholic liver disease varies from alcoholic steatosis to hepatitis, to cirrhosis. The above do not necessarily occur sequentially and may occur independently of each other. The short-term ingestion of 80 g of ethanol/day produces mild reversible hepatic changes. Chronic intake of 50–60 g/day may cause severe injury.

Alcoholic Steatosis (Fatty Liver)

- The severity of fatty change is roughly proportional to the duration and degree of alcohol intake.
- Patient presents with hepatomegaly and mildly increased serum bilirubin and alkaline phosphatase.

Pathogenesis

Hepatocellular steatosis results from

- Impaired assembly and secretion of lipoproteins.
- Increased peripheral catabolism of fat to release free fatty acids (FFA) into the circulation and increased delivery of FFA to liver.
- Generation of **excess reduced NAD (NADH)** by two major enzymes of alcohol metabolism, namely, alcohol dehydrogenase and acetaldehyde dehydrogenase. Decreased NAD^+ inhibits catabolism (oxidation) of fatty acids and leads to increased lipid synthesis and accumulation of fat in hepatocytes.

Pathology

- The liver is enlarged, soft, yellow and greasy.
- Micro- and macrovesicular fat droplets (clear vacuoles) are seen in the cytoplasm of hepatocytes.
- Steatosis starts from the centrilobular region.
- There is minimal accompanying inflammation and absence of fibrosis.

Alcoholic Hepatitis

- Fifteen to twenty years of alcohol intake predispose an individual to alcoholic hepatitis. It usually presents suddenly after a bout of excessive drinking with malaise, anorexia and upper abdominal discomfort, elevated ALT and AST with AST/ALT ratio > 1.
- Unlike fatty liver which reverses completely after alcohol withdrawal and proper nutrition, alcoholic hepatitis may sometimes persist and progress to cirrhosis.

Pathogenesis

- Acetaldehyde, a major metabolic intermediate of alcohol, induces lipid peroxidation and acetaldehyde–protein adduct formation, which disrupts cytoskeletal and membrane proteins.
- Alcohol directly affects microtubule organization, mitochondrial and membrane functions.
- Reactive oxygen species (ROS) generated during oxidation of ethanol by microsomal ethanol oxidizing system can damage membrane and proteins.
- ROS are also generated by neutrophils infiltrating the liver.
- Under normal circumstances glutathione is transported from the cytoplasm to the mitochondria where it neutralizes the ROS. There is impairment of this transport in alcoholic liver disease leading to mitochondrial dysfunction by ROS.
- In the intestine, alcohol causes release of endotoxin (lipopolysaccharide or LPS) from the gram-negative flora which enters portal circulation to induce production of proinflammatory cytokines (TNF-alfa, IL-6 and TGF-alfa) from kupffer cells. This causes hepatocellular injury/damage.

Pathology

The liver is enlarged, yellow but firm on account of fibrosis. The following microscopic features are seen:

- **Hepatocyte swelling and necrosis:** Ballooning and necrosis of single and small groups of hepatocytes.
- **Mallory–Denk bodies or Mallory hyaline:** Tangled skeins of intermediate filaments visible as dense, eosinophilic inclusions in the perinuclear zone cytoplasm of hepatocytes. Also seen in Wilson disease, chronic cholestatic syndromes and hepatocellular tumours.
- **Neutrophil infiltration:** Present around degenerating hepatocytes particularly those with Mallory bodies.
- **Fibrosis:** Initially pericellular **(chicken wire fence pattern)**, sinusoidal and perivenular; with prolonged bouts of alcohol intake; periportal fibrosis may also be seen.

Alcoholic (Laennec or Portal) Cirrhosis

It is the irreversible end stage of alcoholic liver disease which entails a diffuse loss of architecture with fibrosis and nodule formation.

Pathogenesis

Activation of Stellate cells and portal fibroblasts eventually progresses to extensive central–central, central–portal and portal–portal fibrosis.

Pathology

- Liver is yellow, fatty and enlarged in the initial stages and becomes brown, shrunken and firm in the later stages.
- Capsular surface shows nodules (hobnail appearance – initially micronodules are seen and they later coalesce to form macronodules to show a mixed pattern).
- There is diffuse loss of normal parenchymal and vascular architecture with formation of regenerating nodules.
- New vascular channels form in the fibrous septae which connect the portal vessels with the terminal hepatic veins.

Q. Write briefly on nonalcoholic fatty liver disease (NAFLD).

Ans. NAFLD is a group of disorders which resemble alcoholic steatohepatitis but occurs in the absence of alcohol intake.

- Important risk factors for development of this entity are obesity, Type II diabetes mellitus and hyperlipidaemia (metabolic syndrome*). Insulin resistance increases lipid accumulation (steatosis). The lipid so formed is dysfunctional leading to decreased production of the lipid hormone 'adiponectin' with a simultaneous increase in inflammatory cytokines like IL-6 and TNF-α. This predisposes the fat-laden hepatocytes to apoptosis and oxidative injury, which in turn is responsible for hepatocellular necrosis and associated inflammation.
- NAFLD is a common incidentally discovered cause for abnormal liver tests (once other causes of liver diseases are excluded).
- NAFLD is broadly divided into two groups:
  (a) Patients with isolated fatty liver disease (80%): These patients are asymptomatic at the time of diagnosis; some have fatigue, malaise and hepatomegaly. They show none or minimal progression to cirrhosis.
  (b) Patients with nonalcoholic steatohepatitis or NASH (20%): NASH shows a histology identical and to alcoholic hepatitis. Patients with NASH have a much higher propensity to progress to cirrhosis and hepatocellular carcinoma. NASH is a significant contributor to the group 'cryptogenic cirrhosis'.

Q. Write briefly on the aetiology and clinicopathological features of haemochromatosis.

Ans. Haemochromatosis is a condition in which there is excessive iron absorption leading to parenchymal iron overload. It may be hereditary or acquired in nature.

1. **Hereditary haemochromatosis**
   Occurs due to mutations in genes encoding for proteins regulating hepcidin levels, eg, haemochromatosis gene (HFE gene; located on chromosome 6), transferrin receptor (TFR) 2 gene and haemojuvenile (JJV) gene or the hepcidin gene itself. Hepcidin, a hepatocellular protein which has bactericidal activities, is the main regulator of iron absorption and is encoded by HAMP gene. It lowers plasma iron levels and a mutation in either hepcidin gene itself or the genes encoding for the regulatory proteins result in iron overload.

Aetiology

- Mutations in HFE gene and TFR 2 gene lead to the classic adult form of hereditary haemochromatosis.
- Mutations in the HAMP gene or **HJV** lead to a severe form of hereditary haemochromatosis called neonatal haemochromatosis.

**Source:* WHO criteria for defining metabolic syndrome:
Any one of the following:
- Diabetes mellitus or
- Impaired glucose tolerance or
- Impaired fasting glucose or
- Insulin resistance

And two of the following:
- Blood pressure > 140/90 mm Hg
- Dyslipidaemia (Triglycerides > 169.5; HDL cholesterol < 0.9 mmol/L in males and < 1 mmol/L in females)
- Central obesity (waist–hip ratio > 0.90 in males and > 0.85 in females or body mass index > 30 kg/m^2)
- Microalbuminuria (urinary albumin > 20 mcg/min and albumin/creatinine ratio > 30 mg/gm)

- The most common form of the disease is inherited as an autosomal recessive disorder characterized by mutations in the HFE gene that regulates the levels of hepcidin, the iron hormone produced by liver, which inhibits iron absorption.
- Hepcidin levels are reduced in all known forms of haemochromatosis leading to increased absorption of dietary iron over years.
- Ninety percent of the patients are males (females are protected by the iron loss in menstruation and pregnancy).
- Excessive iron can be directly toxic to host tissues by the following mechanisms:
  - Lipid peroxidation by iron-mediated free radical reactions
  - Interaction of iron and reactive oxygen species generated by the iron directly with DNA leading to cell injury and predisposition to hepatocellular carcinoma
  - Stimulation of Ito cells/hepatic stellate cells to produce more collagen

Pathology

- The excess iron deposited in various tissues results in damage to liver, pancreas, heart, pituitary gland and skin.
- Pancreas show diffuse interstitial fibrosis and parenchymal atrophy with haemosiderin deposits in the acinar as well as islet cells (the latter causing diabetes).
- Heart is enlarged with haemosiderin deposits in the myocardial fibres (causing arrhythmias and cardiomyopathy).
- Haemosiderin deposits in the synovium leads to acute synovitis.
- Testes are small and atrophic (leading to loss of libido and infertility).

Clinical Features

- The total body iron ranges between 2 g (normal is 4 g).
- Presents in men over 40 years.
- Fully developed cases show a triad of
  (i) Micronodular cirrhosis
  (ii) Diabetes mellitus **(bronze diabetes)**
  (iii) Skin pigmentation (attributed mainly to excess melanin production and partly to haemosiderin deposits)
- Other manifestations include loss of libido, testicular atrophy, spider nevi, loss of body hair, jaundice and ascites, heart failure and cardiac arrhythmias.
- It is associated with a high incidence of hepatocellular carcinoma.

2. **Acquired (secondary) haemochromatosis (also called haemosiderosis)**
   Develops secondary to:
   (a) Chronic anaemias:
      (i) Thalassaemia major
      (ii) Sideroblastic anaemia
   (b) Exogenous iron overload:
      (i) Multiple blood transfusions
      (ii) Repeated iron injections
      (iii) Prolonged oral iron intake (including African iron overload or Bantu siderosis)
   (c) Chronic liver diseases
   (d) Porphyria cutanea tarda

Note: In secondary iron overload, iron accumulates in Kupffer cells rather than hepatocytes (accumulation in hepatocytes typically occurs in hereditary haemochromatosis).

Q. Write briefly on the aetiology and clinicopathological features of Wilson disease (hepatolenticular degeneration).

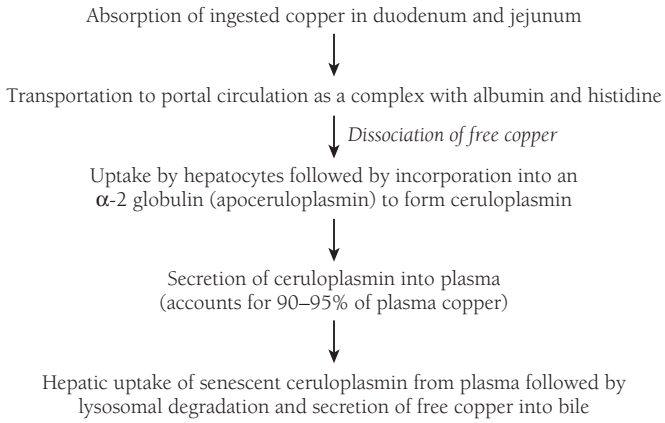
Ans. The following are the salient features of Wilson disease:

Aetiology

- Hereditary disorder with autosomal recessive inheritance

- Due to mutation in ATP7B, a gene located on chromosome 13 (encodes for ATPase metal iron transporter, localized to Golgi region of hepatocytes; the deficiency of which impairs copper excretion into bile)

Normal Copper Physiology (Flowchart 15.3)



FLOWCHART 15.3. Normal copper physiology.

Wilson disease is characterized by the following abnormalities:

- Failure of secretion of ceruloplasmin in plasma
- Failure of biliary copper excretion causing its accumulation in the body

Copper causes toxic liver injury by:

- Inducing formation of free radicals
- Binding to sulphydryl groups of cellular proteins
- Displacing other metals from hepatic metalloenzymes

Clinicopathological Features

- Presents between 5 and 30 years
- The excess copper is deposited in various tissues resulting in damage to:

Liver

- Fatty change
- Acute and chronic hepatitis with hepatocytic ballooning and presence of 'Mallory–Denk bodies'
- Massive liver cell necrosis
- Cirrhosis

Brain (basal ganglia)

Basal ganglia show atrophy and cavitation leading to **neuropsychiatric manifestations:**

- **Neurological manifestations:** Movement disorders, especially resting tremors. Less commonly spasticity, rigidity, chorea, dysphagia and dysarthria may be seen.
- **Psychiatric manifestations:** Bizarre behavioural disturbances similar to schizophrenia, manic-depressive psychosis and neurosis.

Eyes: Kayser–Fleischer rings (green to brown deposits of copper in the Descemet membrane in the limbus of cornea). Kayser–Fleischer rings may be associated with **'sunflower cataracts'.**

Others: RBCs show haemolysis, deposits of copper in kidneys may cause renal tubular damage and in the skeleton may cause osteoporosis.

Investigations

- Slit-lamp examination of the eyes for Kayser–Fleischer rings

- Low serum ceruloplasmin levels (<20 mg/dL)
- High urinary copper excretion (>100 mcg/day; most specific test)
- High hepatic copper content (>250 mcg/g of dry tissue)
- Serum copper levels can be raised, low or normal so are of no diagnostic use

Q. Write briefly on the aetiology and clinicopathological of α-1 antitrypsin deficiency.

Ans. The following are the salient features of α-1 antitrypsin deficiency:

- α-1 antitrypsin is an α-1 globulin produced by the liver. It comprises 90% of the α-1 globulins.
- It is a serine protease inhibitor (Pi), which inhibits the protease enzymes, particularly neutrophil elastase, cathepsin G and proteinase 3, to prevent breakdown of elastin and collagen by them.
- It is encoded by a gene located on chromosome 14, which is extremely polymorphic and more than 70 forms have been identified. The most commonly encountered forms of α1-antitrypsin are
  - PiM (medium)
  - PiS (slow)
  - PiZ (very slow)
- **PiMM** is the normal phenotype, while the phenotype **PiZZ** gives low α-1 antitrypsin concentrations (less than 10% of normal levels).
- α-1 antitrypsin deficiency may lead to **liver** and **pulmonary diseases** (cirrhosis and emphysema, respectively).

Clinicopathology

- In neonates, α-1-antitrypsin deficiency produces hepatitis and cholestatic jaundice.
- In adults, the patient may present with any of the following:
  - Chronic hepatitis
  - Cirrhosis
  - Hepatocellular carcinoma (in 2–3% patients with a PiZZ phenotype)
  - Emphysema
- Most cases of α-1-antitrypsin deficiency are characterized by presence of intrahepatic round-to-oval PAS-positive cytoplasmic globular inclusions.

Q. Name the two main autoimmune disorders of intrahepatic bile ducts. Write briefly on the clinicopathological features of both.

Ans. The two main autoimmune disorders of intrahepatic bile ducts are

1. Primary biliary cirrhosis (PBC)
2. Primary sclerosing cholangitis (PSC)

PBC

Salient Features

- Shows nonsuppurative destruction of small- and medium-sized intrahepatic bile ducts followed by cirrhosis.
- Large intrahepatic ducts and the extrahepatic biliary structures are not involved.
- Occurs predominantly in women between 30 and 70 years, all of who do not present with cirrhosis, indicating that the name is a misnomer.
- It often occurs in association with Sjögren syndrome, scleroderma and thyroid disease.
- Ninety-five percent are AMA-positive, 20% ANA-positive and 60% ANCA-positive.

Pathology

- Early lesions show dense lymphocytic and plasma cell infiltrate around small bile ducts in the portal tracts.
- Late lesions show chronic granulomatous inflammation destroying the interlobular bile ducts **(florid duct lesion),** resulting in fibrosis and later cirrhosis of the liver.
- In both early and late stages, there is marked hepatomegaly, contrary to other end-stage liver diseases which show a small shrunken liver. This is probably due to the minimal hepatocytic loss and extensive regeneration, typical of PBC.

PSC

Salient Features

- PSC is an immune-mediated chronic cholestatic disease characterized by progressive concentric periductal (onion skin) fibrosis and destruction of extrahepatic and large intrahepatic bile ducts. It has the following features:
- Median age is 30 years.
- Patient presents with fatigue, pruritis, jaundice, increased ALP levels and other features of chronic cholestatic liver disease.
- Patchy involvement of the biliary tree results in characteristic '**beading**' appearance of the affected segment during a retrograde cholangiogram.
- Commonly coexists with **inflammatory bowel disease, pancreatitis** and **retroperitoneal fibrosis.**
- Sixty-five percent patients are ANCA-positive.
- Cholangiocarcinomas may develop in 10–15% cases.

Pathology

- Obstruction of intrahepatic bile ducts leads to proliferation of bile ductules, inflammation and necrosis of adjacent periportal hepatic parenchyma and cholestasis.
- Large bile ducts show periductal fibrosis that obliterates the lumen leaving a solid cord-like scar with a few inflammatory cells.
- Primary biliary cirrhosis and primary sclerosing cholangitis eventually lead to end-stage liver disease (liver becomes hard and finely granular and shows yellow-green pigmentation).

Q. Differentiate between PBC and PSC.

Ans. The differences between PBC and PSC are summarized in Table 15.4.

TABLE 15.4. Differences between PBC and PSC

S. No.	Feature	PBC	PSC
1	**Average age affected**	50 years	30 years
2	**Gender**	90% females	70% females
3	**Evolution**	Progressive	Unpredictable
4	**Associated conditions**	Sjögren syndrome	Inflammatory bowel disease Pancreatitis
5	**Serology**	95% AMA positivity 50% ANA positivity 40% ANCA positivity	5% AMA positivity 6% ANA positivity 65% ANCA positivity
6	**Radiological features**	Normal	Beaded appearance of the affected segment on a retrograde cholangiogram is diagnostic.
7	**Pathology**	Small- and medium-sized intrahepatic bile ducts are affected. Large intrahepatic ducts and the extrahepatic biliary structures are not involved.	Causes progressive sclerosing destruction of bile ducts of all sizes. Extrahepatic and large intrahepatic bile ducts are mainly involved.

Q. Write briefly on the aetiology, clinical features and morphology of hepatocellular carcinoma (HCC)/hepatoma.

Ans. Salient Features

- HCC accounts for 80–90% of all liver cancers.
- Occurs more often in men than women; presents with abdominal pain, malaise, weight loss and palpable/radiologically detected lesion.
- Most common in Africa and South-East Asia which show a high rate of chronic HBV infection. HCC in these countries occurs earlier (20–40 years) and in half the cases there is no evidence of background cirrhosis. In the Western countries, increase in the incidence of HCC is attributed to hepatitis C; it manifests after 60 years and in 90% cases shows background cirrhosis.

Predisposing Factors

- Chronic hepatitis B and C infections
- Aflatoxin toxicity (a fungal toxin present in moulds and grains and produced by the fungus *Aspergillus flavus*)
- Alcoholic cirrhosis
- Primary biliary cirrhosis
- NAFLD and metabolic syndrome
- Haemochromatosis
- α-1 antitrypsin deficiency
- Wilson disease
- Anabolic steroids, thorotrast and arsenic
- Oestrogens and androgens

Note: Aflatoxin and alcohol synergize with HBV and HCV and even cigarette smoking to increase the risk of HCC.

Pathogenesis

Presence of structural/numerical chromosomal aberrations in HCC possibly attributed to:

1. Repeated cycles of death, inflammation and active hepatocyte replication (regeneration) in chronic hepatitis induce genomic instability in hepatocytes.
2. Point mutation or overexpression of cellular genes, ie, β-catenin and loss of heterozygosity of tumour suppressor genes, ie, P53. Recent studies indicate that IL-6/JAK/STAT pathway may have a role (IL-6 is shown to suppress hepatocytic differentiation and increase their proliferation by enhancing the function of the transcription factor HNF-α).
3. Defects in DNA repair.
4. HBV-X gene may have some oncogenic potential.
   Precursor lesions of HCC
   (a) HCC is thought to arise from **mature hepatocytes** and **progenitor cells** called ductular cells and oval cells.
   (b) Dysplasias in the liver can be classified as small cell change (cells show high nuclear–cytoplasmic ratio, nuclear hyperchromasia and pleomorphism) and large cell change (large pleomorphic cells which may show multiple nuclei). The former is thought to be directly premalignant whereas the latter is considered directly premalignant only in the presence of hepatitis B and is otherwise just identified as a marker for increased risk of HCC.
   (c) High-grade dysplastic nodules are definitely premalignant and show cytological atypia. The premalignant potential of low-grade dysplastic nodules is uncertain though they have been shown to be clonal. They do not show cytological or architectural atypia.

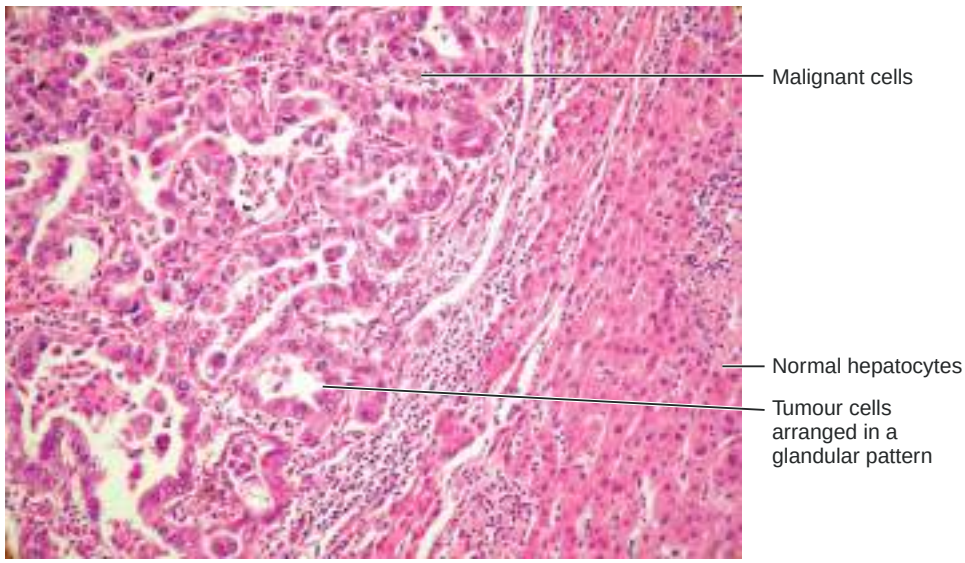


FIGURE 15.6. Photomicrograph of HCC showing large, well-differentiated, polygonal cells with central nuclei and frequent mitotic figures. The cells are arranged mainly in an acinar pattern (H&E; 400×).

Morphology

- HCCs can be **solitary (unifocal), multicentric (multifocal)** or **diffuse infiltrating.**
- Classic HCC shows large, well-differentiated, polygonal cells with central nuclei and frequent mitotic figures. The cells are typically arranged in a trabecular pattern. Acinar pattern (Fig. 15.6), cord-like arrangement and nests of tumour cells may also be seen.
- Poorly differentiated lesions show sheets of less-differentiated cells interspersed with anaplastic tumour giant cells. Areas of haemorrhage and necrosis are common.
- These lesions invade adjacent vascular structures or abdominal structures and may metastasize to lungs, adrenals, lymph nodes or bone.
- A distinct histological variant, termed **fibrolamellar carcinoma** (5% of all HCCs) occurs with relatively high frequency in children and young adults. It presents as a single hard scirrhous nodule. This tumour subtype shows large polygonal well-differentiated cells arranged in nests, cords or large islands separated by bundles of acellular dense collagen. The fibrolamellar variant is generally associated with a more favourable prognosis.

Investigations

- Markedly increased or rising levels of alpha-fetoprotein and CEA
- Ultrasonography/CT scan of abdomen
- Hepatic artery angiography shows 'tumour blushes'
- Aspiration (FNAC) or biopsy confirms the diagnosis

Q. Write briefly on metastatic liver disease.

Ans. Metastasis to liver is more common than primary malignancy. The most common sources of hepatic metastasis are GIT, breast, lung and pancreas. In addition to these, most other cancers can metastasize to the liver (leukaemias, lymphomas, melanomas, etc.). The liver is enlarged with the presence of a single or multiple metastatic nodules. The nodules appear as umbilicated masses (umbilication is due to necrosis or haemorrhage in the centre as the tumour outgrows its blood supply).

Q. Write briefly on the clinicopathological features of pyogenic liver abscess.

Ans. Clinicopathological features of pyogenic liver abscess

- Bacteria reach the liver by:
  - Vascular seeding (via portal blood in appendicitis, diverticulitis and perforated bowel and via hepatic artery in systemic bacteraemia).
  - Ascending cholangitis
  - Direct extension from a contiguous focus of infection, like subphrenic abscess
  - Penetrating injury
- Solitary abscess is usually located in the right lobe of liver and results from direct extension of infection and trauma.
- Multiple abscesses are seen in elderly patients, and are usually due to ascending cholangitis.
- *E. coli*, *Klebsiella* species, anaerobic streptococci and Bacteroides are the common causative organisms.
- Clinical features include fever, right upper quadrant pain and tender hepatomegaly.
- Small lesions respond to antibiotics whereas larger lesions need surgical drainage.

BILIARY TRACT

Q. Write briefly on the aetiopathogenesis, clinicopathological features and complications of gallstones.

Ans. Gallstones affect 10–20% of adult males and 30–40% of adult females.

Types

1. Cholesterol (contain more than 50% of crystalline cholesterol monohydrate); more common in the west
2. Pigment (main constituents are bilirubin and calcium); more common in Asians

Cholesterol Stones

Risk Factors

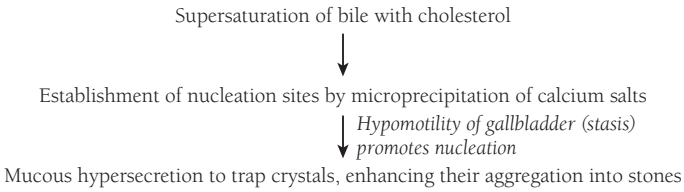
- Demography: Western more than Asians
- Advancing age
- Female gender, oral contraceptives, pregnancy, obesity and rapid weight reduction
- Reduced gallbladder motility
- Inborn disorders of bile acid metabolism
- Hyperlipidaemia syndromes

Salient Features

- They occur in two forms:
  - **Pure cholesterol stones:** Rare, large, solitary, spherical and finely granular, with a yellow glistening radiating crystalline internal structure.
  - **Mixed cholesterol stones:** They account for the majority of stones found clinically and are composed predominantly of cholesterol, but also contain variable amounts of bilirubin and calcium salts. Most often, these stones are multiple and 85% of them are radiolucent and cannot be seen on regular X-ray films.

Pathogenesis (Flowchart 15.4)

- Cholesterol which is normally water insoluble becomes water soluble when it complexes with bile salts and lecithins secreted into bile.
- When excess cholesterol accumulates in the bile, it supersaturates (does not remain dissolved anymore and precipitates out). This results in its nucleation into solid cholesterol monohydrate crystals.



FLOWCHART 15.4. Pathogenesis of cholesterol stone formation.

Pigment Stones

Risk Factors

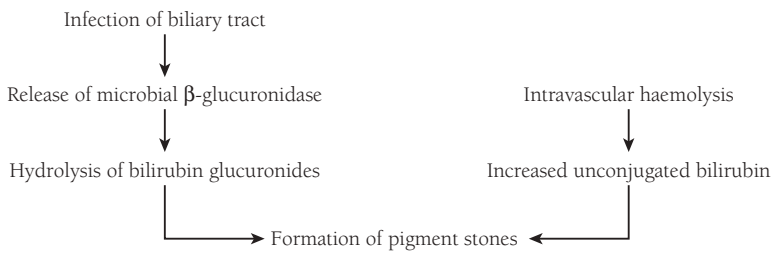
- Demography: Asian more than Western
- Chronic haemolytic syndromes
- Biliary infection
- Gastrointestinal disorders: Ileal disease and cystic fibrosis with pancreatic insufficiency

Salient Features

Pigment stones are either black or brown:

- **Black stones** are composed of **calcium bilirubinate, phosphate, carbonate and very little cholesterol.** These are usually multiple, small and friable and form in chronic haemolytic anaemias, such as sickle cell anaemia or thalassaemia.
- **Brown stones** are composed of **calcium bilirubinate, calcium salts of palmitate and stearate and cholesterol but do not contain calcium phosphate or carbonate**. Usually seen in bacterial infections causing deconjugation of bilirubin and in prolonged biliary stasis and are laminated soap like, greasy.

Pathogenesis (Flowchart 15.5)



FLOWCHART 15.5. Pathogenesis of pigment stone formation.

Cholecystitis

Inflammation of gallbladder is labelled cholecystitis. It is of two types—acute and chronic.

1. **Acute cholecystitis**
   ***Salient features*:**
   - Females are more often affected than males.
   - Associated with gallstones in 90% cases; some cases may be acalculus in origin (acalculus cholecystitis is usually encountered in severely ill patients.
   - Secondary bacterial infection may follow obstruction in some cases—*Escherichia coli* is the most common pathogen.
   - Typically manifests with acute onset of pain in the right upper quadrant, fever and leukocytosis; mild jaundice is present in 20% of cases due to the small stones in the common bile duct.

   ***Pathology*:**
   - Gallbladder is enlarged, distended, tense and assumes a red, violaceous to green-black colour, there may be fibrinous or suppurative exudate on the serosa.

- Stones are often present in the neck of the gallbladder or the cystic duct.
- Gallbladder lumen is filled with cloudy or turbid bile with or without admixed pus.
- When the contained exudate becomes pure pus, the condition is called **empyema.**
- In severe cases, gallbladder is transformed into a **green-black necrotic organ (gangrenous cholecystitis).**
- Histologically, the wall shows oedema, vascular congestion and neutrophilic infiltrate.

2. **Chronic cholecystitis**

***Salient features*:**

- May follow repeated attacks of acute cholecystitis or develop without any history of previous attacks.
- Clinically, it presents with recurrent attacks of colicky epigastric or right upper quadrant pain, nausea, vomiting and intolerance to fatty food.
- Usually associated with gallstones in the lumen or presence of biliary gravel (thick viscous bile with micro-concretions).
- Chronic acalculus cholecystitis causes symptoms and morphological alterations similar to chronic calculus cholecystitis.

***Pathology*:**

- Serosa is dull and opaque and may show adhesions.
- Mucosa is oedematous, focally ulcerated or indurated.
- Gallbladder may be contracted, of normal size, or enlarged.
- Microscopic examination reveals chronic inflammatory infiltrate in the wall (Fig. 15.7), subepithelial and subserosal fibrosis and extension of mucosal sinuses into the muscularis **(Rokitansky–Aschoff or RA sinuses).**

Complications of Cholecystitis

- **Cholangitis:** Bacterial super infection leading to local spread
- **Sepsis:** Bacterial dissemination by blood
- **Subhepatic abscess:** Perforation leading to subhepatic abscess or bacterial peritonitis
- **Empyema:** Accumulation of pus in an obstructed gallbladder due to secondary bacterial infection
- **Emphysematous cholecystitis:** Due to infection by gas-forming organisms, eg, clostridia

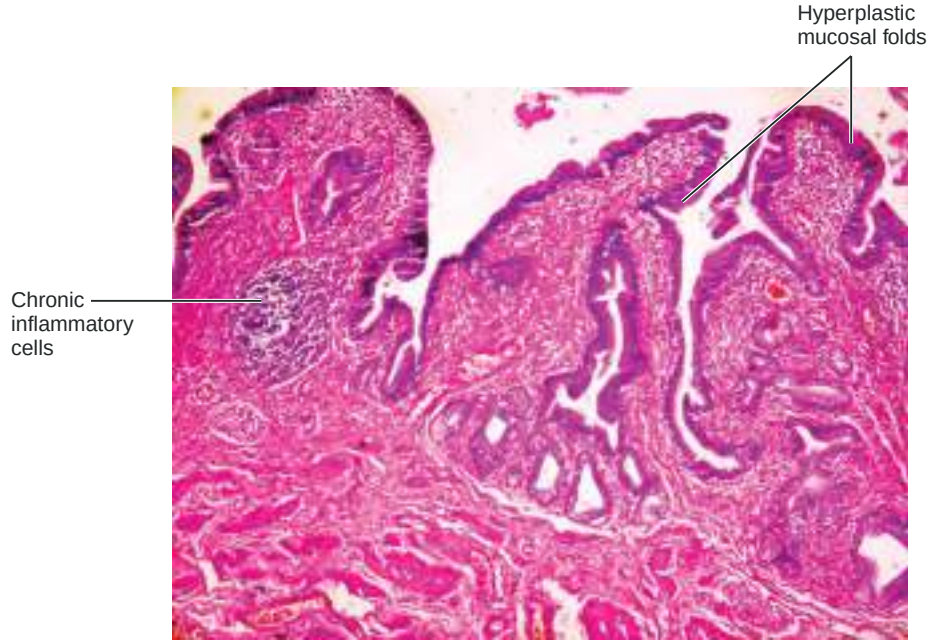


FIGURE 15.7. Section from chronic cholecystitis showing gall bladder wall infiltrated by chronic inflammatory cells (H&E; 100X).

- **Cholecystoenteric fistula:** Formation of a fistula between the gallbladder and the intestine
- **Gallstone ileus:** May follow the impaction of a gallstone in the intestine
- **Porcelain gallbladder:** Scarring of the wall, combined with dystrophic calcification that transforms the gallbladder into a porcelain-like vessel, visible on standard X-ray films
- **Xanthogranulomatous cholecystitis:** The gall bladder may be shrunken and show a markedly thickened wall as a result of rupture of an RA sinus. Sections from the wall show chronic inflammatory infiltrate with foamy histiocytes (xanthoma cells).

Q. Write briefly on carcinoma of gallbladder.

Ans. The average age of presentation of carcinoma of gallbladder is 65 years. It is associated with gallstones in up to 90% of cases; porcelain gallbladder is a high-risk condition.

- Patient presents with abdominal pain, jaundice, anorexia, nausea and vomiting.
- Preoperative diagnosis is based on finding abnormalities in the gallbladder wall on imaging studies.
- Grossly carcinoma of the gallbladder may be exophytic or more commonly infiltrative in nature; the latter usually appears as diffuse thickening of the wall of the gallbladder.
- Most carcinomas are adenocarcinomas (90%); few are squamous or adenosquamous carcinomas (10%) that arise from areas of squamous metaplasia in chronic cholecystitis and cholelithiasis.

Q. Write briefly on cholangiocarcinomas.

Ans. Cholangiocarcinoma, a malignancy arising from the biliary tree, is the second most common tumour of the liver after HCC. It has the following clinicopathological features:

- It usually presents in the fifth to seventh decades and has a male to female ratio of 1:1.
- Risk factors include liver fluke infestation, hepatolithiasis (intrahepatic gallstone formation), PSC, fibrocystic disease of the biliary tree, hepatitis B and C, NAFLD and exposure to thorotrast.
- Biliary intraepithelial neoplasias (BillN) are known precursors of cholangiocarcinomas, which are mainly adenocarcinomas with biliary differentiation.
- May be extrahepatic or intrahepatic; extrahepatic cholangiocarcinomas (two-third of these tumours) may develop at the hilum (called **Klatskin tumours**) or more distally.
- The prognosis is poor.

PANCREAS

The pancreas is located in the retroperitoneal space caudal to the stomach. It extends horizontally from the duodenum on the right to the spleen on the left. It has three parts:

- **The head** of the pancreas is lying in the duodenal loop in close contact with the wall of this part of the intestine.
- **The body** of the pancreas is lying over the aorta and the vena cava.
- **The tail** of the pancreas abuts onto the spleen.
- Pancreas is a mixed exocrine–endocrine organ.
- The exocrine pancreatic tissue (consists of acini and ducts) accounts for 98% of the total mass.
- Endocrine parts (islets of Langerhans) are microscopic structures that are more numerous in the tail.
- The terminal portion of the main pancreatic duct (duct of Wirsung) enters the muscular portion of the duodenal wall, where it meets with the common bile duct forming a common biliary-pancreatic duct that enters the duodenum at the ampulla of Vater.
- In some persons, the pancreatic duct enters the duodenum separately from the common bile duct.
- An accessory pancreatic duct (duct of Santorini), emptying into the duodenum, is found in many persons.

Cells Forming the Islets of Langerhans

- Alpha cells: Secrete glucagon (increases blood glucose)
- Beta cells: Secrete insulin (lowers blood glucose)
- Delta cells: Secrete somatostatin (inhibit the secretion of other islet hormones)
- Delta-1 cells: Secrete vasoactive intestinal polypeptide (VIP), which regulates and stimulates the motility of intestines
- PP cells: Secrete pancreatic polypeptide (stimulate gastric secretion and inhibit intestinal mobility)

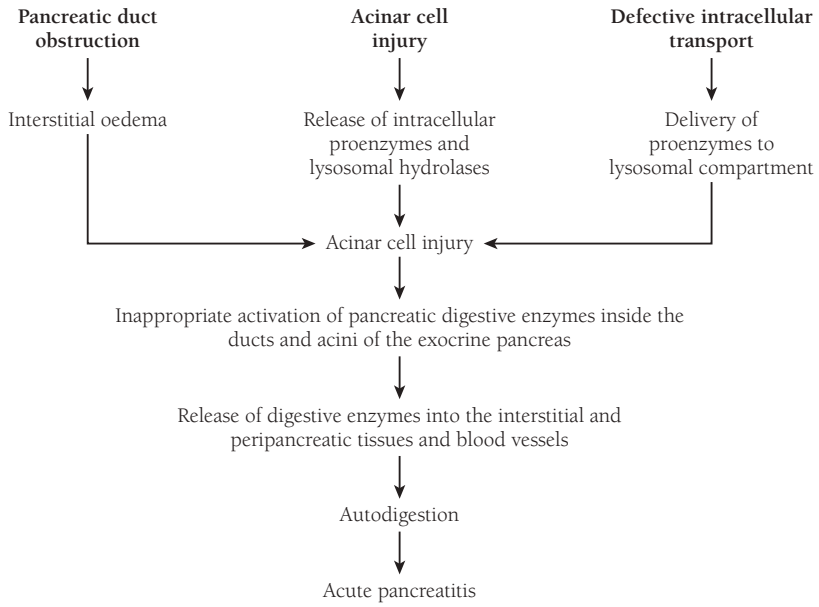
Q. Write briefly on the clinicopathological features of acute pancreatitis.

Ans. Acute pancreatitis is defined as acute inflammation of the pancreas usually resulting from injury to the exocrine pancreas.

Causes

- **Metabolic:** Hyperlipoproteinaemias, hypercalcaemia, alcoholism, drugs (eg, diuretics, azathioprine and mercaptopurine)
- **Genetic:**
  - Inherited mutations in genes encoding pancreatic enzymes or their inhibitors, eg, SPINK1 (serine peptidase inhibitor Kazal type 1) which is an inhibitor of trypsin.
  - Hereditary pancreatitis with trypsinogen mutation is an autosomal dominant disease caused by a mutation in PRSS1 gene that affects a site on trypsinogen molecule required for cleavage of trypsin, leading to continuous activation of other digestive proenzymes and development of pancreatitis.
- **Mechanical:** Trauma (seat-belt injury), gallstones, injury during endoscopic procedures like endoscopic retrograde cholangiopancreatography (ERCP) or perioperative injury
- **Vascular:** Shock, embolus and polyarteritis nodosa
- **Infections:** Mumps, coxsackie virus, mycoplasma pneumoniae, EBV and CMV
- **Idiopathic pancreatitis:** Occurs without any obvious cause and accounts for 10% of the cases, and is the most common cause of pancreatitis (after alcohol and biliary disease)

The mechanisms underlying genesis of acute pancreatitis are summarized in Flowchart 15.6.



FLOWCHART 15.6. The mechanisms underlying the genesis of acute pancreatitis.

Pathology

- Damage to microvasculature leads to oedema.
- Fat necrosis by lipases causes fat cells to become indistinct and ghost like (loss of internal structure). The entire field appears bluish due to the deposition of calcium salts. These areas appear chalky white on gross examination.
- Acute inflammatory reaction
- Destruction of pancreatic parenchyma (pancreatic acini and ducts)
- Destruction of blood vessels with resulting haemorrhage.
- Ascites is found in severe cases. The fluid is turbid, brownish yellow, or blood tinged.

Occurs in Two Forms

- **Milder form:** Mostly, there is interstitial oedema with mild inflammation. Focal mild necrosis of acinar cells may sometimes be seen. This form occurs in terminally ill patients, various forms of shock and after prolonged operations. It is recognized by mild elevation of pancreatic enzymes in the blood, is self-limiting and usually resolves spontaneously.
- **Acute necrotizing/haemorrhagic pancreatitis:** May be life-threatening. Caused by enzyme-mediated destruction of pancreatic and peripancreatic tissue. Neutrophils invade the necrotic tissue. In later stages, neutrophils are replaced by macrophages and the entire area undergoes fibrosis.

Clinical Features

- Sudden onset of severe abdominal pain usually in the left upper quadrant of the abdomen; may radiate to the back
- Nausea and vomiting, fever, sweating, tachypnea and tachycardia followed by peripheral vascular collapse

Laboratory Findings

- **Neutrophilic leukocytosis:** Increase in neutrophil count
- **Serum amylase:** It is a sensitive marker for acute pancreatitis in the first 24 h; especially, if the elevation is four times above normal values.
- **Urinary amylase:** Amylase is excreted in urine. Amylase levels in urine become elevated from the second day onwards and may remain elevated for 7–10 days. This test has little specificity and sensitivity.
- **Serum lipase:** It appears little later than amylase in blood, but it is more specific.
- **Trypsin:** This enzyme has the highest specificity and sensitivity for pancreatic injury, but its measurement requires the use of a radioimmunoassay, which is not available in all hospitals.
- **Hypocalcaemia:** Due to precipitation of calcium in the areas of fat necrosis. If persistent indicates a poor prognosis.
- **X-rays:** Plain X-rays are important to exclude perforation of an ulcer (visible air under the diaphragm), and **CT scan** aids in demonstrating the enlarged pancreas and ascites (fluid if analysed biochemically shows increased amount of pancreatic enzymes).

Complications

- **Shock:** It is multifactorial, but mostly due to increased vascular permeability caused by the action of pancreatic enzymes.
- **DIC:** Endothelial injury caused by pancreatic enzymes in circulation leads to the formation of platelet and fibrin thrombi in small vessels.
- **ARDS:** It is a manifestation of shock.
- **Renal failure:** It is mostly a consequence of shock.
- **Pseudocyst formation:** Massive necrosis leads to liquefactive necrosis of the tissue. The necrotic area becomes walled off by granulation tissue, which transforms later into a fibrous scar. The cyst contains fluid full of pancreatic enzymes.

- **Abscess:** Infection superimposed on pancreatic necrosis leads to abscess formation. It is associated with high mortality.
- **Haemorrhagic ascites.**
- **Subcutaneous fat necrosis:** Foci of fat necrosis develop and are related to the action of lipolytic enzymes that have entered the circulation.
- **Chronic pancreatitis:** Most patients with acute pancreatitis recover if treated appropriately. Persistence of inflammation leads to chronic pancreatitis.

Q. Write briefly on the clinicopathological features of chronic pancreatitis.

Ans. Chronic pancreatitis is characterized by chronic inflammation with fibrosis leading to a progressive loss of pancreatic function. The pancreas is reduced in size and often showed calcification.

Causes

- Chronic alcohol abuse
- Cystic fibrosis of the pancreas
- Familial chronic pancreatitis and 'tropical chronic pancreatitis'
- Idiopathic

Clinical Features

- Persistent upper abdominal pain radiating to the back, often precipitated by alcohol
- Malabsorption due to pancreatic insufficiency—steatorrhoea, vitamins A, D, E and K deficiency
- Diabetes mellitus
- X-ray may show calcifications and distorted ducts can be visualized by ERCP

Pathology

- Persistent chronic inflammation composed of lymphocytes, macrophages and plasma cells.
- Fibrosis, calcification and intraductal concretions
- Loss and atrophy of acini, with partial preservation of ducts and islets of Langerhans
- Cystic dilatation of ducts distal to narrowing by fibrous tissue

Q. Classify pancreatic tumours. Write briefly on the clinicopathological features of pancreatic carcinoma (infiltrating ductal carcinoma of pancreas).

Ans. ***Classification of Pancreatic Tumours***

1. Ductal tumours (90%)
2. Islet cell tumours (5%)
3. Acinar tumours (2%)
4. Others

Pancreatic Carcinoma

- Pancreatic carcinoma accounts for 6% of all cancer deaths
- Most patients are old (>60 years)
- Males and females are almost equally affected

Aetiopathogenesis

Molecular aspects of pancreatic carcinogenesis
Telomere shortening and mutations in the oncogene KRAS and tumour suppressor genes SMAD4, TP53, BRCA2 and CDKN2a are implicated. Telomeric shortening and KRAS mutations are early events followed by inactivation of SMAD4, TP53 and BRCA2.

Risk Factors

- Cigarette smoking
- Chronic pancreatitis and diabetes mellitus
- Diet high in fat and low in vegetables

Clinical Features

- Pain (persistent and usually progressive)
- Anorexia and weight loss
- Jaundice
- Migratory superficial thrombophlebitis (Trousseau sign)

Morphology

- Indurated white mass (may be confused with chronic pancreatitis)
- Head is involved most often (60%), but it may occur in any part of the pancreas.
- Histologically, most tumours are adenocarcinomas with a desmoplastic stroma.
- Invasive ductal cancers are thought to arise from non-invasive intraductal lesions called pancreatic intraepithelial neoplasia (PanIN).
- Perineural invasion, lymphatic and blood-borne metastasis are common. Prognosis is extremely poor, and only 10% of patients survive 2 years.

Q. Enumerate and describe pancreatic tumours of endocrine origin.

Ans. Tumours of endocrine origin are classified according to the secretory function of their cells:

1. Insulinomas (beta cell tumours)
2. Glucagonomas (alpha cell tumours)
3. Gastrinomas
4. Somatostatinomas
5. VIPomas
6. PPomas

Salient Features of Endocrine Tumours of the Pancreas

- Endocrine tumours are rare.
- They are generally composed of cords and nests of uniform cells with round nuclei and moderate amount of cytoplasm (identical to intestinal or bronchial carcinoids).
- Most are low-grade malignant tumours (except insulinomas, which are usually benign).
- Benign tumours may show 'endocrine atypia' and cannot be distinguished from the malignant tumours on the basis of histology alone. Metastasis is the only definitive sign that a tumour is malignant.
- Endocrine tumours secrete hormones that produce typical syndromes.

16

Diseases of the Kidney and Lower Urinary Tract

NORMAL STRUCTURE

The kidneys are paired, bean-shaped, retroperitoneal organs each weighing about 150 g in the adult male and 135 g in the adult female. They are typically 10–12 cm in length, 5–7 cm in width and 2–3 cm in thickness. The renal artery, vein, lymphatics and the ureters are located in the renal hilum which is the centre of the concave area of the kidney. The upper and lower poles of each kidney lie opposite to the twelfth thoracic vertebra, and the third lumbar vertebra, respectively. Right kidney is slightly lower due to the presence of liver. The renal capsule **is a smooth, transparent, fibrous membrane that is normally easily removable. It protects the organ and is surrounded by perirenal fat which further cushions the kidneys.**

The **cut surface** of the bisected kidney shows a pale outer region, the **cortex** and a darker inner region, the **medulla** (Fig. 16.1**).** The cortex contains all the glomeruli and 85% tubules (mainly proximal convoluted tubules). The medulla is divided into 8 to 18 conical masses, the **renal pyramids,** the bases of which lie along the corticomedullary junction and the apices extend into the renal pelvis (the collecting system of the kidney) to form **papillae.** The tip of each papilla has 10 to 25 small openings of the distal ends of the **collecting ducts (of Bellini).** The renal cortex is about 1.5 cm in thickness and covers

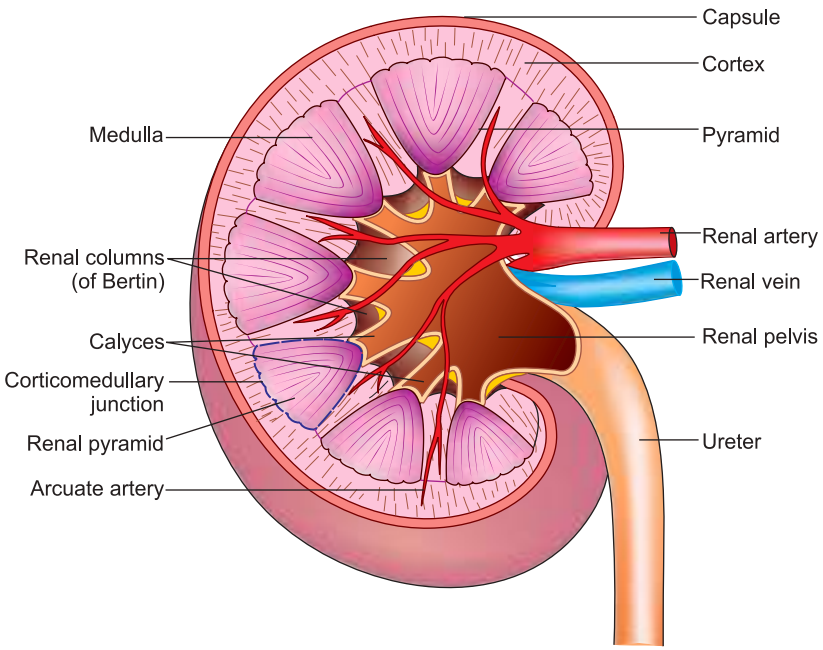


FIGURE 16.1. Schematic representation of the cut surface of kidney.

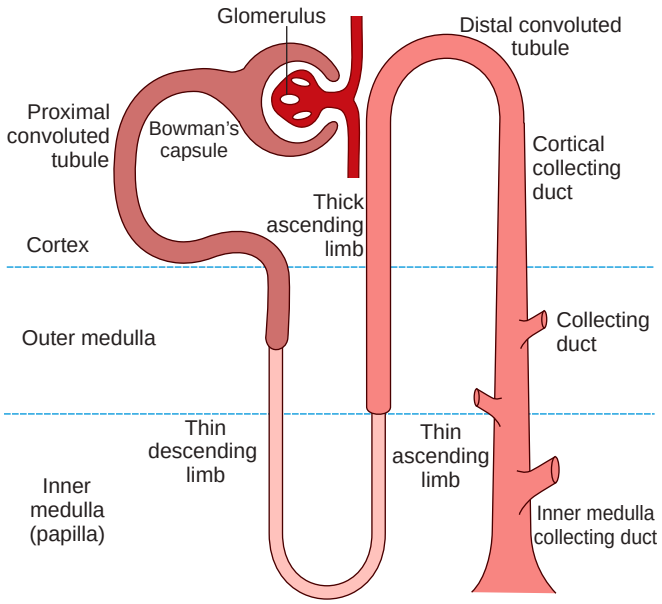


FIGURE 16.2. Parts of a nephron.

the base of each renal pyramid to extend downward between the individual pyramids to form the **renal columns of Bertin.** The ureter on entering the kidney dilates to form the **renal pelvis** which is lined by transitional epithelium and forms 2–3 outpouchings, the **major calyces.** From each of the major calyces, several **minor calyces** extend toward the papillae of the pyramids.

The main unit of parenchyma of each kidney is the **nephron.** There are about 1–4 million nephrons in each kidney. Each nephron contains 5 major subunits, the dilated 'glomerulus with the Bowman capsule', 'the proximal convoluted tubule or PCT', 'the thin and thick loop of Henle', 'the distal convoluted tubule' or DCT and the 'collecting ducts' (Fig. 16.2).

The **glomerulus** is a bulbous structure invaginated by a capillary network which is surrounded by a double-layered epithelial capsule called the **Bowman's capsule.** The inner layer enveloping the capillary tuft is called the **visceral layer** and the outer layer is called the **parietal layer.** Between the visceral and the parietal epithelial layers is a cavity called **Bowman's space.** The large area of the capillary network makes the glomerulus an efficient filtration unit. Each nephron has a **vascular pole** and a **urinary pole.** The **vascular pole** is where the afferent arteriole enters and the efferent arteriole leaves. The PCT begins at the **urinary pole.** The inner side of the glomerular capillary wall is lined by a thin layer of fenestrated endothelial cells which rest on the glomerular basement membrane (GBM). The GBM is constituted by collagen, laminin, fibronectin, proteoglycans and glycoproteins and has three layers:

(a) Lamina rara externa on the external side
(b) Lamina densa in the middle
(c) Lamina rara interna on the internal side

Any abnormality in the glomerular epithelial cells or the above-mentioned three layers may disturb the barrier to filtration of macromolecules.

The glomerulus is supported by mesangial cells with surrounding mesangial matrix material. The external side of the capillary wall is lined by the visceral epithelial cells which rest on the lamina rara externa. These cells have foot-like extensions and are therefore also called podocytes (podo-foot). The podocytes have 20–30 nm wide spaces between them to allow filtration (Fig. 16.3).

The **main function of the glomerulus** is filtration from the capillaries to the Bowman's space. Normal glomerular filtration rate is about 125 mL/min. The glomerular filtrate is identical to plasma in composition except it lacks cells and protein.

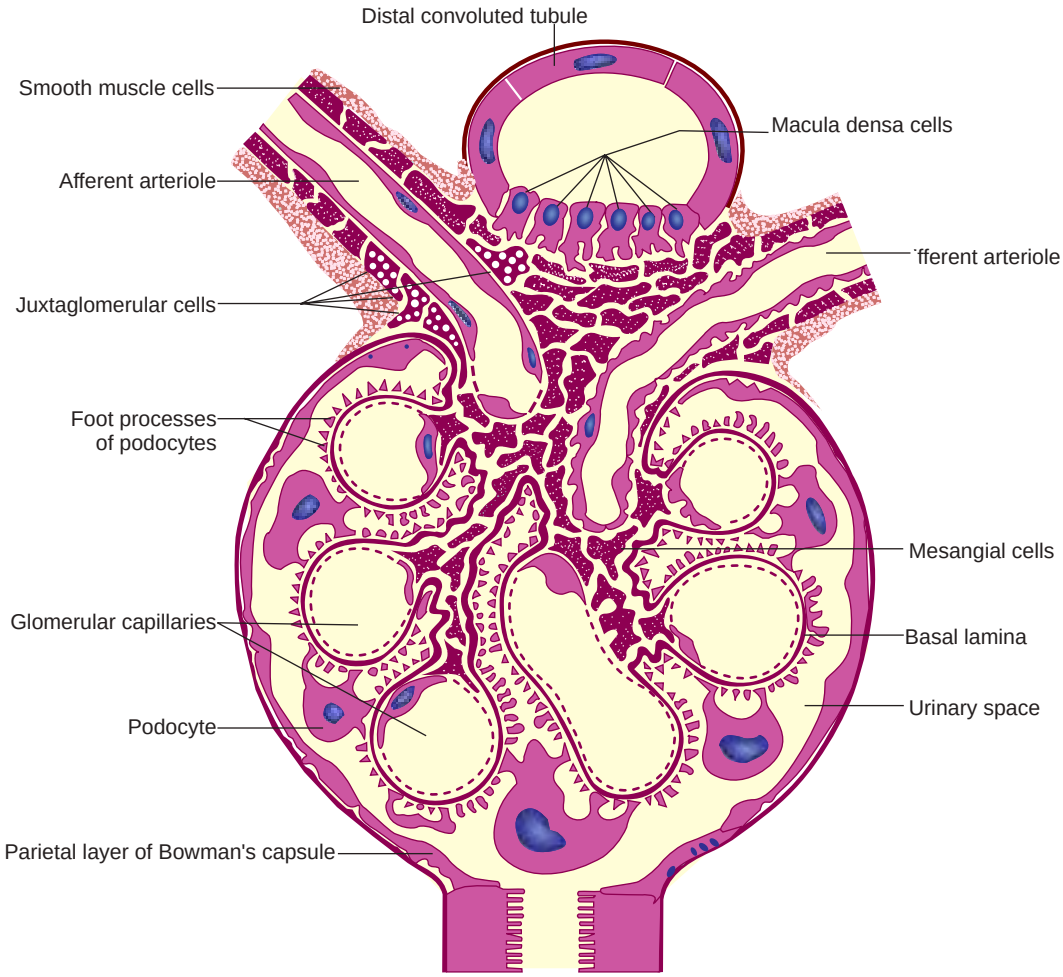


FIGURE 16.3. Schematic representation of a renal glomerulus.

Glomerular filtration occurs across the following barrier:

1. Fenestrated endothelial cells lining the capillaries
2. Glomerular basement membrane (GBM) associated with endothelial cells
3. Pores between the foot processes of the podocytes

GFR is influenced by

1. Blood pressure and blood flow
2. Obstruction to urine outflow
3. Hormonal regulation
    1. Renin—angiotensin
    2. Aldosterone
    3. Antidiuretic hormone (ADH)
    4. Atrial natriuretic peptide (ANP)

Tubules

The bulk of the renal substance is formed by tubules. The tubular epithelium varies in different parts of the nephron depending upon their function.

1. **PCT:** It is the first part of the glomerulus responsible for active reabsorption of filtered sodium, potassium, glucose, amino acids and proteins, bicarbonate, phosphate, calcium and uric acid as well as passive reabsorption of water. It arises at the urinary pole

and is lined by cuboidal cells with a brush border (presence of microvilli), acidophilic granular cytoplasm and central nuclei.

2. **Loop of Henle:** The PCT continues as the straight part of loop of Henle. The loop of Henle begins near the corticomedullary junction; it is U shaped and has a thin descending and a thick ascending portions. The thin portion is lined by flat epithelium with nuclei projecting into the lumen. The thick portion is identical in structure to the DCT and ends at the corticomedullary union.
3. **DCT:** The thick part of loop of Henle becomes tortuous, enters the cortex and continues as the DCT. It is lined by flatter cells which are smaller in size as compared to cells lining the PCT, are less acidophilic and do not have a brush border. The lumina of the distal tubules are larger due to the smaller size of the lining cells. DCT touches the vascular pole of the renal corpuscle of its nephron close to the point of entry of the afferent arteriole. Here, the lining epithelium gets modified to become columnar with closely packed nuclei (thereby appearing darker). This area is called the **macula densa.**
4. **Collecting tubules:** The collecting ducts join to form the larger straight ducts called the papillary ducts of Bellini. Collecting tubules form the major bulk of the medulla. The smaller ducts are lined by cuboidal epithelium; however, as they dip into the medulla the lining epithelium becomes columnar. The cytoplasm of the cells is uniformly pale staining.

Juxtaglomerular Apparatus (JGA)

The JGA is located in the vascular pole of the glomerulus and has three parts:

1. Juxtaglomerular cells—These are epithelioid cells with granular cytoplasm located in the media of the afferent arteriole and secrete rennin.
2. Macula densa
3. Extraglomerular mesangial or Lacis or Polkissen cells—Lightly staining cells whose function is not clearly understood.

Vascular Supply

The kidneys receive approximately 20% of the cardiac output from the **paired renal arteries** which enter into the renal hilum. The anterior half of the kidney can be divided into upper, middle and lower segments, each supplied by a **segmental branch of the anterior division of the renal artery**. The posterior half of the kidney is divided into apical, posterior and lower segments, each supplied by **branches of the posterior division of the renal artery**. The **segmental branches** branch into **interlobar arteries,** which travel between the major calyces to branch further into **arcuate arteries.** The arcuate **arteries** run between the cortex and medulla across the bases of the renal pyramids. They then radiate into **interlobular** arteries, extend into the cortex of the kidney to finally become **afferent arterioles**, each of which supplies a single glomerulus. From the glomerulus arise the **efferent arterioles.** The efferent arterioles supply the **peritubular capillary plexus** which anastomoses with the capillary plexus of another nephron. Some of the terminal branches of the interlobular arteries become **perforating radiate arteries**, which supply the renal capsule.

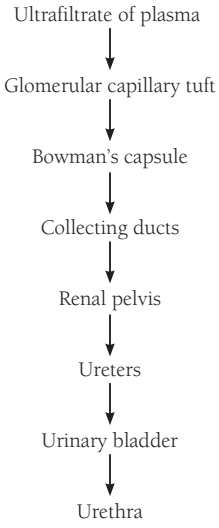
FUNCTIONS OF THE KIDNEY

1. Maintenance of electrolyte levels and acid–base balance
2. Regulation of blood pressure and maintenance of salt and water balance
3. Removal of water soluble wastes from the blood, eg, urea and ammonia and reabsorption of water, glucose and amino acids
4. Production of hormones like calcitriol, erythropoietin and rennin

URINE FORMATION

- Kidney maintains water and electrolyte balance and contributes to acid–base homeostasis.
- Composition of urine varies with water, salt and protein intake.

- Variability in composition may create a practical problem in the timing of collection of urine specimens.
- Timed urinary collections are preferred to random specimens.
- In a normal adult, 25% of cardiac output (>1 L of blood) perfuses two kidneys each minute.
- The essential steps in the formation of urine are as follows (Flowchart 16.1):



FLOWCHART 16.1. Formation and flow of urine.

1. Filtration of substances from blood into Bowman's capsule (glomerular filtrate formation)
2. Reabsorption of some of the filtered substances back into the blood stream
3. Secretion of substances from blood into tubule

Composition of Urine

- Most of the solute is urea and sodium chloride.
- Protein intake affects nitrogen excretion, which is mainly as urea.
- Uric acid, creatinine, amino acids, ammonia, traces of proteins, glycoproteins, enzymes and purines account for the remaining nitrogen excreted.
- Potassium, sulphates, sulphides, cysteine, mercaptans, small amounts of sugars (pentoses), oxalic acid, citric acid, pyruvate, trace amounts of cholesterol and metals are present.
- Also present are hormones, eg, ketosteroids, oestrogens, aldosterone, gonadotrophins, catecholamines, ascorbic acid, along with trace amounts of bilirubin, haemoglobin and porphyrins seen.
- **Microscopic constituents (formed elements)** of urine include RBCs, leukocytes, renal tubular epithelium, transitional and squamous epithelium and physiologic casts.
- **Changes in urine on standing:**
  - Colour changes: Due to breakdown of chromogen
  - Odour changes: Due to bacterial growth/decomposition
  - Increased turbidity: Due to bacteriuria, crystals and precipitation of amorphous material
  - Falsely ↓ pH: Due to breakdown of urea to ammonia by bacteria/loss of CO_2
  - Falsely ↓ or absent glucose: Due to bacterial utilization
  - Falsely negative ketone: Due to volatilization of acetone; breakdown of acetoacetate by bacteria
  - Decreased bilirubin: Destroyed by light and oxidized to biliverdin
  - Disintegration of cells/casts (especially in hypotonic and alkaline urine)

Q. Enumerate and describe the different methods of collection of urine.

Ans. Collection of urine

- Urine should be collected in a clean and dry container.
- It should be freshly voided (collected within 2 h of voiding).
- If analysis is delayed, specimens should be refrigerated or preserved.
- The desired volume of urine to be collected is 50–60 mL as the minimum quality of urine required for estimation of specific gravity by a urinometer is 30 mL.

Types of urinary specimens:

1. **Voided specimens:** Suitable for chemical and microscopic examinations.
   (a) **First morning specimen** is best for proteins, nitrites, microscopic examination and cytology (preferred because of large volume and concentrated urine).
   (b) **24-h urine specimen** is optimum for quantitative protein/sugar/urobilinogen estimation.
   ***Method of collection*:** Patient is carefully instructed to empty bladder at 8 a.m. and discard the urine. All subsequent samples are collected, including that at 8 a.m., the following morning. Urine should be pooled and thoroughly mixed prior to analysis.
   (c) **Midstream sample** is best for bacterial examination.
   ***Method of collection*:** Using complete aseptic precautions, separate labia, expose urethral orifice (labia kept separated throughout the collection), clean the area surrounding the meatus with soap balls and then the meatus itself, allow the initial stream of urine to drain and catch the subsequent midstream sample. In males, glans is exposed, thoroughly cleaned with a mild antiseptic solution and dried, foreskin retracted, midstream sample collected.
2. **Catheterized specimens may be obtained by:**
   (a) Ureteric catheterization
   (b) Urethral catheterization

Catheterized specimen is best for cytological examination as it is free of seminal fluid, vaginal cells, inflammatory cells and microorganisms.

Q. Enumerate the different chemical and cytological preservatives for urine.

Ans. Reliable results are obtained when a fresh specimen has been properly refrigerated up to 48 h. Chemical or cytological preservatives should be added if the analysis is delayed any further.

Chemical Preservatives

- Mineral acids/vitamin C lower pH
- Boric acid inhibits bacterial multiplication
- Benzoic acid, phenol, thymol, toluene, chloroform, formaldehyde and mercury compounds prevent bacterial growth
- Sodium fluoride decreases glycolysis in cells and bacteria and is therefore used for glucose estimation in a concentration of less than 0.5 g per 3–4 L urine.

Cytology Preservative

Equal volume of 50% alcohol.

Q. Write in detail on examination of a urine specimen.

Ans. Examination of a urinary specimen entails physical, chemical and microscopic examination.

Physical Analysis of Urine

Colour

Normal colour of urine is due to three pigments, namely, **urochrome, urobilin, uroerythrin.** The following colour changes can be seen in different clinical conditions:

- Pale urine: high fluid intake
- Dark urine: dehydration
- Cloudy urine: presence of mucus, precipitation of phosphates or urates (turbidity disappears on addition of acetic acid), bacterial growth, sperms and prostatic fluid
- Red urine: presence of haemoglobin, RBCs, myoglobin, porphyrins, beets and menstrual contamination
- Milky urine: pyuria, lipiduria and chyluria

Odour

- Normal urine has a faint aromatic odour. Bacterial contamination leads to an ammoniacal, fetid odour.
- Characteristic odour is noted in some conditions, eg, mousy in phenylketonuria, sulphuric smell in cysteine decomposition, faecal smell in gastrointestinal-bladder fistulae and other abnormal smells with some medications (vitamin B_6); and diet (asparagus).

Volume

- Normal: 1200–1500 mL in 24 h.
- Polyuria: More than 2000 mL of urine in 24 h (seen in excessive fluid intake, diuretic therapy, chronic kidney disease, diabetes insipidus, mental disorders, DM and primary aldosteronism)
- Nocturia: More than 500 mL of urine with a specific gravity of less than 1.018 at night.
- Oliguria: Less than 500 mL of urine in 24 h (seen in dehydration, acute glomerulonephritis, shock, toxic nephropathy, obstruction to urinary flow)
- Anuria: Complete suppression of urine formation

Specific Gravity (SG)

- Normal: 1.016–1.022
- Low SG (hyposthenuria): SG less than 1.007 (seen in excessive fluid intake, diuretic therapy, chronic kidney disease, diabetes insipidus)
- Low fixed SG (isosthenuria): SG fixed at 1.010 (seen in chronic renal failure), as the concentrating power of kidney is lost due to tubular damage
- Increased SG: SG greater than 1.022 (seen in dehydration, glycosuria, renal artery stenosis, heart failure due to decreased blood flow to the kidneys, inappropriate antidiuretic hormone secretion and proteinuria)

Methods of Estimation of Specific Gravity

1. Urinometer
   (a) Fill three-fourth of the cylinder of the urinometer with urine (minimum volume required 15 mL). Gently lower the urinometer in the cylinder and set the urinometer in spinning motion (should be free floating; not touching sides; there should be no bubbles).
   (b) Read bottom of meniscus
   (c) Calibrate with:
      (i) Temperature—0.001 for each 3°C above or below 20°C
      (ii) Protein concentration—0.003 for every 1 g/100 mL protein
      Check calibration every day by measurement of specific gravity of distilled water (which is 1.000).
2. Refractometer: Requires only a few drops of urine. It is used to measure the refractive index. Refractometers are instruments that can relate density of a solution to specific gravity. They work on the principle that light passing from a transparent medium of one

density to a medium of another density will change its velocity and therefore the direction in which the beam of light is moving.

3. An indirect colorimetric method for estimating specific gravity is available on reagent strips ('urine dipsticks'): This method uses a pad that contains a complex, pretreated electrolyte that undergoes a pH change based on the ionic concentration of the urine. This change results in a change of colour of the pad. This estimate of specific gravity is rapid, simple and requires no special equipment.

pH

- pH is the ability of kidneys to maintain normal hydrogen ion concentration in plasma and extracellular fluid. Metabolic activity produces nonvolatile acids, eg, sulphuric acid, phosphoric acid, hydrochloric acid, pyruvic acid, lactic acid, citric acid and ketones. These are excreted and bicarbonates reabsorbed.
- Normal pH: 4.6–8
- Measured by reagent strips (recommendations: protect the strips from moisture and heat, store in a cool dry area, do not refrigerate, check for discoloration and check manufacturer's directions).

Chemical Analysis of Urine

Chemical examination of urine includes testing for proteins, glucose, ketones, bile derivatives and blood. Most common abnormalities detected on chemical examination of urine are

- **Glycosuria:** Causes include DM, renal glycosuria, pregnancy, alimentary glycosuria, intravenous infusion of glucose and increased intracranial tension.
- **Proteinuria:** Kidney diseases (like nephritic syndrome, nephrotic syndrome, tuberculous nephritis, renal cell carcinoma and renal vein thrombosis), muscular exertion, high fever, heavy metal poisoning and orthostatic albuminuria) can lead to proteinuria.
- **Ketonuria:** Metabolic abnormalities such as diabetes, glycogen storage diseases, starvation, fasting, high protein, or low carbohydrate diets, prolonged vomiting and hypermetabolic states such as fever, pregnancy, or lactation are common causes of ketonuria. In nondiabetic persons, ketonuria may occur during acute illness or severe stress.

Microscopic Analysis of Urine

- Centrifuge 10/12/15 mL of urine at 450 g for 5 min
- Remove supernatant leaving behind a few drops
- Mix sediment with a drop or two of the supernatant and resuspend
- Smear and examine

RBCs

- Normal: 0–2 cells/HPF or 3–12 cells/µL.
- Appear as faint, colourless circles/shadow cells (due to dissolution of haemoglobin).
- Hypertonic urine shows crenation of RBCs (may be confused with yeast cells but yeast cells show budding. Also, on adding a few drops of acetic acid, RBCs lyse, but yeast cells do not).
- Distorted RBCs are called dysmorphic erythrocytes (when more than 20% RBCs appear distorted; the RBCs are regarded as renal in origin).
- **Causes of hematuria include:**
  1. Lesions of the urinary tract
     - Kidney: Polycystic kidney, hereditary nephritis, tuberculosis, acute nephritic syndrome, renal tumours (RCC and Wilms tumour), infarction, pyelonephritis, IgA nephropathy and trauma
     - Ureter: Ureteric calculi, papilloma or carcinoma
     - Urinary bladder: Rupture, cystitis, tuberculosis, transitional cell carcinoma (TCC), calculi and *Schistosoma haematobium* infection
     - Prostate: Prostatitis, nodular hyperplasia prostate (NHP) and carcinoma prostate
     - Urethra: Rupture, urethritis, stricture, calculus and TCC

2. Systemic disorders: Blood disorders and collagen diseases
3. Drugs: Salicylates, cyclophosphamide and anticoagulants

WBCs

- More than 20/HPF abnormal
- More than 30/HPF indicative of acute infection
- Adding 2% acetic acid yields better nuclear morphology
- Presence of leukocyte casts is suggestive of renal infection or involvement.

Epithelial Cells

Squamous, transitional and round cells may be seen in the urinary sediment.

Casts

Casts are cylindrical structures with rounded edges composed of Tamm–Horsfall protein secreted by tubular cells. They usually appear in the urine in renal diseases.

- Hyaline casts are the most frequently occurring casts in urine. Hyaline casts can be seen in even the mildest renal disease. They are colourless, homogeneous, transparent and usually have rounded ends. Up to 0–2/low power field are considered normal.
- Red cell casts indicate renal haematuria. Red cell casts may appear brown to almost colourless and are usually diagnostic of glomerular disease.
- White cell casts are present in renal infection (pyelonephritis) and in noninfectious inflammation. The majority of white cells that appear in casts are neutrophils.
- Granular casts almost always indicate significant renal disease. However, granular casts may be present in the urine for a short time following strenuous exercise. Granular casts that contain fine granules may appear grey or pale yellow in colour. Granular casts that contain larger coarse granules are darker. These casts often appear black because of the density of the granules.
- Epithelial casts are rarely seen in urine because the renal disease that primarily affects the tubules is infrequent.
- Waxy casts result from the degeneration of granular casts. Waxy casts have been found in patients with severe chronic renal failure, malignant hypertension and diabetic disease of the kidney. Waxy casts appear yellow, grey or colourless. They frequently occur as short, broad casts, with blunt or broken ends and often have cracked or serrated edges.
- Fatty casts are seen when there is fatty degeneration of the tubular epithelium, as in degenerative tubular disease. Fatty casts also result from nephrotic syndrome, lupus and toxic renal poisoning.

Q. Write briefly on Bence Jones proteinuria.

Ans. Bence Jones proteins are dimers of immunoglobulin light chains, normally produced by plasma cells.

- They are sufficiently small to be excreted by the kidney and are characteristically found in the urine of most patients with **multiple myeloma**, **macroglobulinaemias** and **amyloidosis**. They are used for diagnosis of the disease as well as for monitoring the response to treatment.
- Persistent Bence Jones proteinuria may eventually result in renal failure by two mechanisms:
  - Direct toxicity to epithelial cells
  - Cast nephropathy (combination of Bence–Jones proteins with Tamm–Horsfall protein under acidic conditions may form large tubular casts, which obstruct the lumen and also induce peritubular inflammatory reaction)
- Bence–Jones proteins are detected by:
  - Heat coagulation test
  - Immunoelectrophoresis, which is a more sensitive method and detects even minute quantity of the protein

Q. Enumerate renal function tests.

Ans. Renal function tests include

1. **Urine examination**
   (a) Physical and chemical examination

(b) Microscopic examination
(c) Bacteriologic examination

2. **Blood examination**
   (a) Blood urea (BUN)
   (b) Serum creatinine
   (c) Serum electrolytes
   (d) Serum protein
   (e) Serum cholesterol
   (f) Serum uric acid
3. **Renal clearance tests (CT)**
   (a) Tests for glomerular function
      (i) Inulin CT
      (ii) Creatinine CT
   (b) Tests for tubular function:
      (i) Urea CT
      (ii) Paraamino hippuric (PAH) CT
4. **Concentration and dilution tests**
   (a) Concentration test (fluid deprivation test)
   (b) Dilution test (excess fluid intake test)
5. **Others**
   (a) Intravenous pyelography
   (b) Ultrasonography
   (c) Arteriography
   (d) FNAC/renal biopsy

Q. Differentiate between acute and chronic renal failure.

Ans. Differences between acute and chronic renal failure are tabulated in Table 16.1.

TABLE 16.1. Differences between acute and chronic renal failure

Features	Acute renal failure	Chronic renal failure
Definition	• Rapid onset of renal dysfunction; may be reversible • Manifests with oliguria/anuria and increase in urea and creatinine	• Progressive and irreversible deterioration of renal function due to slow destruction of renal parenchyma • Manifests with azotaemia and acidosis
Causes	Prerenal (ischaemia and hypovolaemia), renal (vascular, glomerular and tubular disorders) and post-renal (obstruction in ureters, bladder and urethra)	All chronic nephropathies like chronic glomerular diseases as well as chronic tubulointerstitial diseases
Metabolic acidosis	Poorly tolerated	Well tolerated
Clinical presentation	Three patterns: 1. Syndrome of acute nephritis 2. Syndrome accompanying tubular dysfunction 3. Prerenal syndrome	Four stages: 1. Diminished renal reserve (50% GFR) 2. Renal insufficiency (20–50% GFR) 3. Renal failure (5–20% GFR) 4. End-stage renal disease (<5% GFR)
Urine output	Markedly decreased	Normal
Serum electrolytes		
Calcium	Normal/low	Low
Phosphate	Increased	Markedly increased
Sodium	Decreased	Markedly decreased
Potassium	Increased	Markedly increased
Haemoglobin	Normal	Reduced (normocytic normochromic anaemia; in case of blood loss, microcytic hypochromic anaemia)
Serum parathormone	Normal	Increased
Alkaline phosphatase	Normal	Increased

Q. Enumerate the cystic lesions of kidney.

Ans. Cysts of the kidney include

- Polycystic kidney (adult and infantile type)
- Medullary cystic disease (medullary sponge kidney and nephronophthisis)
- Localized or simple renal cyst
- Multicystic renal dysplasia
- Acquired (dialysis-associated) cystic disease
- Renal cysts associated with tuberous sclerosis
- Calyceal or pyelogenic cyst
- Pelvic cyst
- Perinephric cyst
- Cystic degeneration in tumours

Q. Differentiate between adult and childhood polycystic kidney disease.

Ans. Differences between adult and childhood polycystic kidney disease are tabulated in Table 16.2.

TABLE 16.2. Differences between adult and childhood polycystic kidney disease

Features	Adult	Childhood
Inheritance	Autosomal dominant, caused by a mutation in the genes encoding **polycystin 1 and 2. Defective gene is PKD1 or PKD2**	Autosomal recessive with a mutation in the gene encoding **fibrocystin, ie, PKHD1**
Frequency	More common	Less common
Presentation	• Presents after fourth decade; may be associated with a cystic liver, berry aneurysm, subarachnoid haemorrhage, colonic diverticulii and mitral valve prolapse • Large, multicystic kidney	• Presents in perinatal/neonatal age group with splenomegaly and hepatic fibrosis (oesophageal varices may be seen as a consequence of hepatic fibrosis) • External surface of the kidney is smooth; cut surface shows numerous small cysts in the cortex and medulla (dilated elongated channels at right angles to the cortical surface)
Origin of cysts	May arise from any level of nephron from tubules to collecting ducts; lining variable	Arises from collecting ducts; lined uniformly cuboidal cells
Clinical features	Haematuria, flank pain, hypertension and urinary infection	Bilateral abdominal mass
Outcome	Chronic renal failure begins at age of 40–60 years	• Young infants usually die of hepatic and renal failure • Patients who survive develop congenital hepatic fibrosis

Q. Enumerate the various glomerular syndromes.

Ans. Based on clinical manifestations, renal diseases are classified into the following major glomerular syndromes:

1. **Acute nephritic syndrome:** Haematuria, azotaemia, variable proteinuria, oliguria, oedema and hypertension
2. **Rapidly progressing glomerulonephritis (RPGN):** Acute nephritis, proteinuria and acute renal failure
3. **Nephrotic syndrome:** Proteinuria > 3.5 g/day, hypoalbuminaemia, hyperlipidaemia and lipiduria
4. **Chronic renal failure (CRF):** Azotaemia and uraemia progressing over years
5. **Asymptomatic haematuria/proteinuria:** Haematuria and subnephrotic proteinuria

Q. Classify glomerular diseases.

Ans. Based on clinicopathological features, glomerular diseases are classified broadly into primary and secondary (Table 16.3).

TABLE 16.3. Clinicopathological classification of glomerular diseases

Primary glomerulonephritis	Secondary (systemic diseases with glomerular involvement)	Hereditary nephritis
• Acute proliferative glomerulonephritis • RPGN • Membranous glomerulonephritis • Minimal change disease • Focal segmental glomerulosclerosis • Membranoproliferative glomerulonephritis • Dense deposit disease. • IgA nephropathy • Chronic glomerulonephritis	DM Amyloidosis SLE Polyarteritis nodosa Microscopic polyangiitis Wegener granulomatosis Henoch–Schonlein purpura Bacterial endocarditis	Alport syndrome Fabry disease

Q. Write in detail on the pathogenesis of glomerular injury.

Ans. The various immune mechanisms involved in the pathogenesis of glomerular injury are:

1. **Antibody-mediated injury**
   - (a) **In situ immune complex deposition**
     - (i) Fixed intrinsic tissue antigens:
       - *Good pasture antigen (anti-GBM nephritis)*: Antibody is directed against an intrinsic fixed antigen that is a normal component of the GBM (noncollagenous domain of the alfa-3 chain of collagen type IV). The deposits show a homogeneous, diffuse and linear pattern.
       - *PLA2R antigen (membranous glomerulonephritis)*: Antibody is directed against M-type phospholipase A2 receptor (PLA2R) located on the glomerular epithelial cell membrane. This antigen complex is partially homologous to Heymann antigen found in rats. Granular, interrupted deposits are seen along the subepithelial aspect of the GBM.
       - *Mesangial antigens*
       - *Others*
     - (ii) Planted antigens: These are nonglomerular antigens, which get planted in the glomerulus by interacting with various intrinsic components in the glomerulus, eg,
       - Cationic molecules, which can bind to the glomerular capillary anionic sites.
       - Larger aggregated proteins like IgG which can deposit in mesangium.
       - DNA which has affinity for GBM components.

       Many exogenous (infectious agents and drugs) and endogenous (DNA, immunoglobulins and immune complexes) antigens can act as planted antigens.
   - (b) **Circulating immune complex deposition**
     - (i) Injury is caused by trapping of circulating antigen–antibody complexes within glomeruli because of their physicochemical properties and the prevailing haemodynamics of glomeruli
     - (ii) Subendothelial (rarely subepithelial) granular deposits either along basement membrane or mesangium or both are seen
     - (iii) The antigens involved could be endogenous antigens (DNA and tumour antigens) or exogenous antigens (infectious products)
   - (c) **Cytotoxic antibodies:** Antibodies directed against glomerular cell components can lead to glomerular injury. For example, antibodies against endothelial cell antigen can cause endothelial injury and intravascular thrombosis. Antibody directed against visceral epithelial cell antigen can cause proteinuria.

2. **Cell-mediated glomerular injury:** Cell-mediated immune reactions in the form of delayed hypersensitivity may be involved in causing glomerular injury.
3. **Activation of alternative complement pathway**
   (a) Direct activation of alternative complement pathway by some polysaccharides, endotoxins or IgA aggregates deposited in glomeruli may cause glomerular injury.
   (b) In membranoproliferative glomerulonephritis II (MPGN II), a circulating antibody termed C3 nephritic factor (C3NeF) binds to C3 convertase, favouring persistent splitting of C3 into C3a and C3b, thus activating the alternative pathway and resulting in hypocomplementaemia.
4. **Secondary pathogenic mechanisms:** Neutrophils, macrophages, complement, platelets and mesangial cells can cause glomerular injury directly or by producing cytokines, chemokines, oxidants and enzymes.
5. **Nonimmunological mechanisms**
   (a) Metabolic glomerular injury (diabetic nephropathy)
   (b) Haemodynamic glomerular injury (systemic hypertension)
   (c) Deposition disease (amyloidosis and cryoglobulinaemia)
   (d) Infectious disease (HIV and hepatitis)
   (e) Inherited (Alport syndrome)

Q. Write briefly on the aetiopathogenesis and clinicopathological features of acute proliferative glomerulonephritis.

Ans. Acute proliferative (post-streptococcal or post-infectious) glomerulonephritis:

- Appears 1–4 weeks after a streptococcal infection of the pharynx or skin.
- Occurs most frequently in children between 6 and 10 years of age but can affect any age.
- Can also be caused by organisms other than streptococcus, eg, *Pneumococcus*, *Staphylococcus* and viral diseases like mumps, measles, chickenpox and hepatitis B and C.

Aetiopathogenesis

- Group A, beta-haemolytic streptococcus has some nephritogenic strains (Types 1, 4 and 12; typing is based on M protein of cell wall).
- Principal antigenic determinants involved in acute post-streptococcal nephritis are thought to be nephritis-associated streptococcal plasmin receptor (NAPIr), streptococcal pyogenic exotoxin B (SpeB; most common) and its zymogen precursor (zSpeB).
- Immune complexes, preformed by the combination of specific antibodies against streptococcal antigens, localize on the glomerular capillary wall and activate the complement system.
- The immunologic system may be activated by streptococcal antigens that adhere to the glomerular structures and act as 'planted antigens' as well as by altered endogenous antigens (GBM proteins altered by streptococcal enzymes).
- Glomerular deposition of immune complexes leads to diffuse proliferation and swelling of glomerular cells as well as infiltration by leukocytes, especially neutrophils.

Clinical Features

- Abrupt onset of malaise and nausea with nephritic syndrome characterized by periorbital oedema (due to mild to moderate proteinuria), oliguria, azotaemia, hypertension and gross haematuria (smoky or cocoa-coloured urine).
- Serum complement levels are low.
- Serum antistreptolysin O antibody levels are elevated in post-streptococcal cases.

Prognosis

- In children, recovery occurs in most cases, some children develop RPGN or chronic renal disease (incidence of chronicity is much less than in adults).
- Fifteen to fifty percent of adults, however, develop end-stage renal disease over a few years to 1–2 decades.

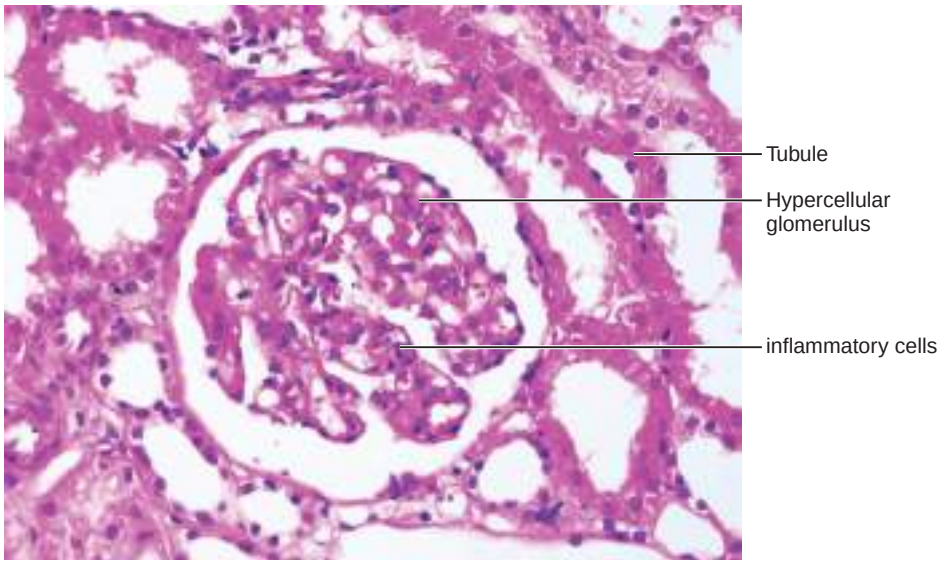


FIGURE 16.4. Microphotograph of acute proliferative (post-streptococcal or post-infectious) glomerulonephritis showing proliferation of endothelial and mesangial cells along with neutrophils and monocytes filtration (H&E; 400X).

Pathology (Fig. 16.4)

- Diffuse (involving the whole kidney) and uniform increase in cellularity of the glomerular tuft due to proliferation and swelling of endothelial and mesangial cells along with infiltration by neutrophils and monocytes.
- Rare cases show necrosis of capillary walls and formation of crescents.
- Electron microscopy (EM) shows deposition of immune complexes as subendothelial, intramembranous and most commonly subepithelial humps. Occasionally, mesangial deposits may be seen.
- Immunofluorescence (IF) shows granular deposits of IgG and complement within capillary walls and mesangium.

Q. Write briefly on RPGN.

Ans. *RPGN* is a clinical syndrome characterized by rapid and progressive loss of renal function. It has features similar to nephritic syndrome but leads to death from renal failure within weeks to months of onset.

Pathogenesis

1. It is caused by systemic diseases as well as diseases localized to the kidney.
2. Regardless of cause, the histological hallmark is the formation of crescents (therefore also called crescentic glomerulonephritis or CrGN; Fig. 16.5). RPGN is of the following types:
   (a) **Type I (anti-GBM antibody type):**
      (i) It has two subtypes—"Renal limited" and "Good pasture syndrome". The former shows linear deposits of IgG and C3 on the GBM.
      (ii) In some of the affected individuals, anti-GBM antibodies also bind to pulmonary alveolar basement membrane to clinically manifest as pulmonary haemorrhages associated with renal failure (Good pasture syndrome).
      (iii) Anti-GBM antibodies can also be detected in the serum and aid in the diagnosis of this disease.

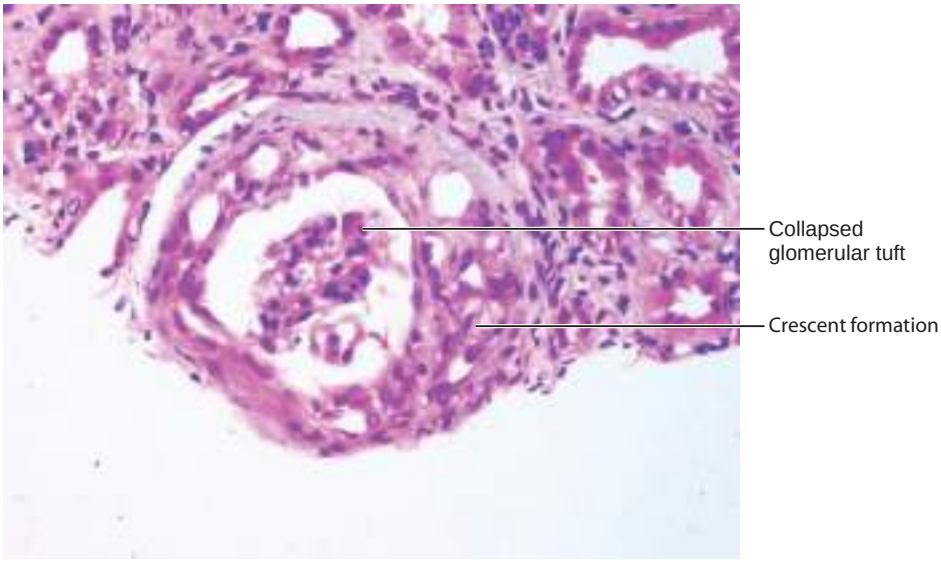


FIGURE 16.5. Microphotograph of RPGN showing cresent formation (H&E; 400X).

(iv) These individuals benefit from plasmapheresis, which removes antibodies from the circulation.
(v) Serum C3 is normal and ANCA is negative.

***Causes*:**
- Idiopathic
- Good pasture syndrome

***Gross morphology*:**
Kidneys are enlarged and pale and show petechial haemorrhages.

***Microscopy*:**
- Segmental necrosis in glomeruli and breaks in the GBM lead to exudation of plasma proteins including fibrin in the Bowman's space.
- Fibrin acts as a stimulus for the proliferation of parietal epithelial cells and infiltration of monocytes into the Bowman's space. This results in formation of crescents because the cells take the shape of the Bowman's space which is crescentic).
- Uninvolved portion of the cells glomerulus shows no proliferation.
- IF shows strong linear staining of IgG and C3 along the GBM.

(b) **Type II (immune complex type) mediated disorder:**
(i) Characterized by granular Ig and C3 deposits
(ii) Serum C3 is low to normal, anti-GBM antibody and ANCA are negative.

***Causes*:**
- Idiopathic
- Post-infectious
- SLE
- Henoch–Schönlein purpura
- IgA nephropathy

***Morphology*:**
- Changes are like Type I disease, however, uninvolved portions of the glomerulus also shows diffuse proliferation and leukocyte infiltration (in post-infectious GN and SLE) or mesangial proliferation (in IgA nephropathy and Henoch–Schönlein purpura).
- EM shows discrete deposits.
- IF demonstrates a granular pattern typical of immune complex disease.

(c) **Type III ANCA-associated (pauci-immune type)**
Lacks immune complex formation or anti-GBM antibodies.

Causes:
- Idiopathic
- Granulomatosis with polyangiitis (Wegener granulomatosis)
- Microscopic polyangiitis

Morphology:
- Same as Type I disease
- Uninvolved segments appear normal without proliferation or inflammation
- In contrast to anti-GBM disease, immunofluorescence studies are negative for immunoglobulins or complement and no deposits are seen on EM

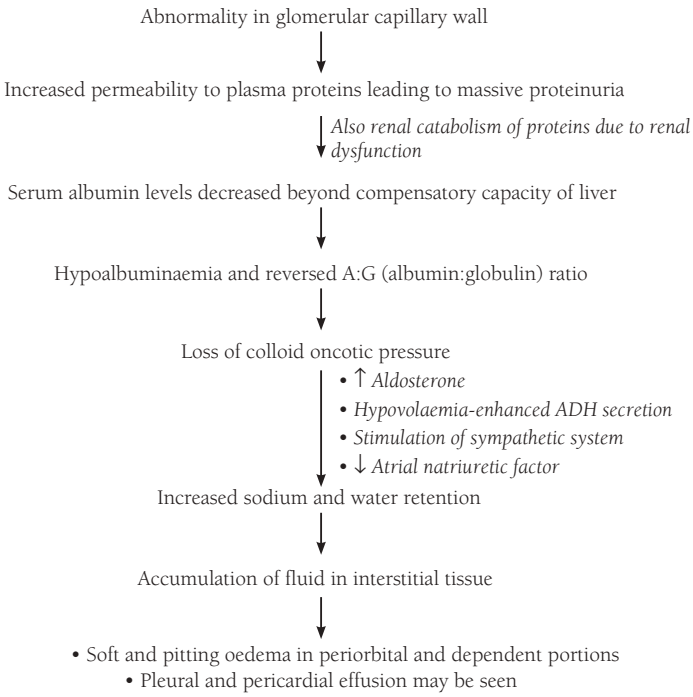
Clinical Features of RPGN:

- Like nephritic syndrome except that oliguria and azotaemia are more pronounced.
- Proteinuria approaches nephrotic range
- Prognosis related to the number of crescents

Q. Define nephrotic syndrome. Describe its pathogenesis. Enumerate its causes and clinicopathological features.

Ans. Nephrotic syndrome is a syndrome complex having the following components:
- Daily loss of >3.5 g of protein (less in children)
- Hypoalbuminaemia with protein levels <3 g/dL
- Generalized oedema/anasarca
- Hyperlipidaemia and lipiduria

Pathogenesis (Flowchart 16.2)



FLOWCHART 16.2. Pathogenesis of manifestations of nephrotic syndrome.

Clinicopathological Manifestations

1. ***Proteinuria which may be***:
   - **Selective** (loss of low molecular proteins, eg, albumin and transferrin)
   - **Nonselective** (high molecular proteins are lost, eg, high molecular weight globulins)

2. ***Hyperlipidaemia which is due to*:**
   1. Enhanced synthesis of lipoproteins in liver
   2. Abnormal transport of circulating lipid particles
   3. Reduced catabolism of lipids

   The lipid-related metabolic abnormalities seen in nephrotic syndrome are
   - Increased cholesterol, triglycerides, VLDL, LDL, LP (a) and apoproteins
   - Decrease in HDL (loss in urine)
   - Lipiduria or oval fat bodies or free fat in urine (lipoproteins reabsorbed by tubular epithelium and then shed with the epithelium when it gets injured and detached)
3. ***Thrombotic complications*:** Renal vein thrombosis due to loss of anticoagulant factors, eg, antithrombin III (AT III)
4. ***Increased susceptibility to infection*:** Due to loss of immunoglobulins in the urine

Causes

- Primary glomerular diseases:
  - Membranous glomerulonephritis
  - Lipoid nephrosis
  - Focal segmental glomerulosclerosis (FSGS)
  - Membranoproliferative glomerulonephritis (MPGN)
  - IgA nephropathy
- Systemic diseases:
  - Diabetes mellitus (DM)
  - Amyloidosis
  - SLE
  - Drugs (gold, penicillamine and street heroin)
  - Infections (malaria, syphilis, hepatitis B and AIDS)
  - Malignancy (carcinoma and melanoma)
  - Miscellaneous (bee-sting allergy and hereditary nephritis)

Membranous Glomerulonephritis

Pathogenesis

- Primary or idiopathic membranous nephropathy is thought to be an autoimmune disease linked to a susceptibility gene like HLA-DQA1. It is caused by an in situ immune reaction involving renal autoantigens (eg, PLA2R) and in some cases, planted antigens. The disease shows resemblance to Heymann nephritis (induced by formation of antibodies to the megalin antigenic complex present in rat podocytes which is the animal counterpart of PLA2R).
- Circulating immune complexes are present in 25% cases.
- Paucity of neutrophils, monocytes and platelets in glomeruli and virtually uniform presence of complement points to direct action of C5b-9 (membrane attack complex), which is thought to activate glomerular epithelial and mesangial cells to release proteases and oxidants, which cause capillary wall injury to lead to protein leakage.

Causes

Idiopathic (primary) in 75% cases; the remaining 25% are labelled **secondary** and may be caused by:

- Malignancies (carcinoma lung/colon/melanoma)
- SLE
- Exposure to inorganic salts (gold and mercury)
- Drugs (penicillamine and captopril), gold and NSAIDs
- Infections (hepatitis B and C, syphilis, schistosomiasis and malaria)
- DM and thyroiditis

***Morphology* (*Fig. 16.6*)**

Light microscopy: The early stages show no abnormality; later, uniform and diffuse thickening of the glomerular capillary wall due to IgG deposits along the epithelial side of the basement membrane is noted.

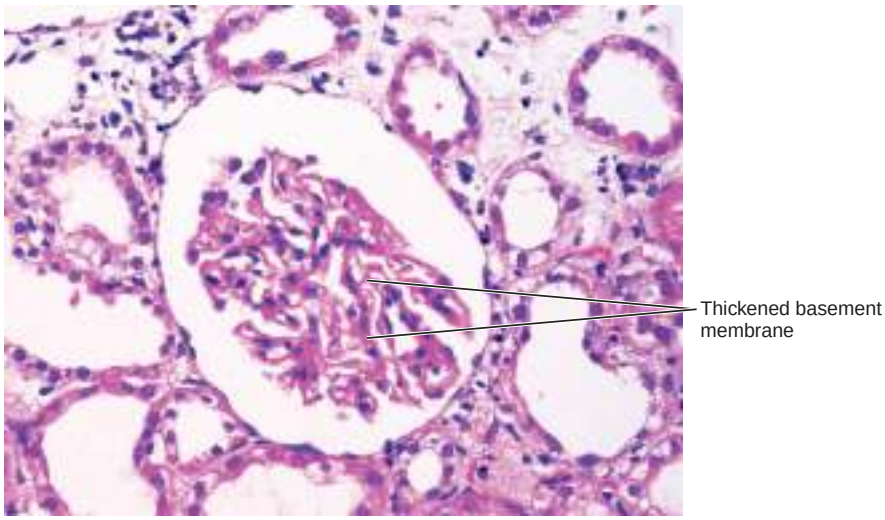


FIGURE 16.6. Microphotograph of membranous glomerulopathy showing uniform and diffuse thickening of the glomerular capillary wall (H&E; 400X).

Electron microscopy: Shows irregular dense deposits of immune complexes between basement membrane and overlying epithelial cells (which have lost their foot processes). Basement membrane material is deposited between these immune complexes as **irregular spikes.** The spikes are best seen by silver stains which colour the GBM but not the deposits. The spikes cover and fuse over the immune deposits resulting in membrane thickening.

Clinical Features

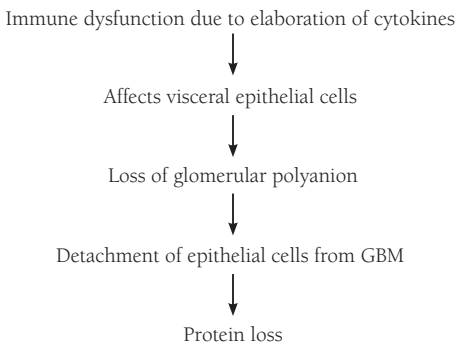
- Insidious onset of nephrotic syndrome in majority; non-nephrotic proteinuria in a few patients.
- Proteinuria is non-selective and responds poorly to steroids (unlike minimal change disease). It persists in > 60% patients and 10% of these go into renal failure.

Lipoid Nephrosis/Minimal Change Disease (MCD)

- Usually occurs in children 2–6 years of age following a respiratory infection or routine immunization and shows a dramatic response to steroids.
- Thought to have an immunologic basis (Flowchart 16.3):

Morphology (Fig. 16.7)

Light microscopy is within normal limits.
Electron microscopy shows effacement of foot processes of visceral epithelial cells, which shows a thin rim of cytoplasm with cytoplasmic vacuolization, swelling and villous hyperplasia.



FLOWCHART 16.3. Pathogenesis of proteinuria in MCD.

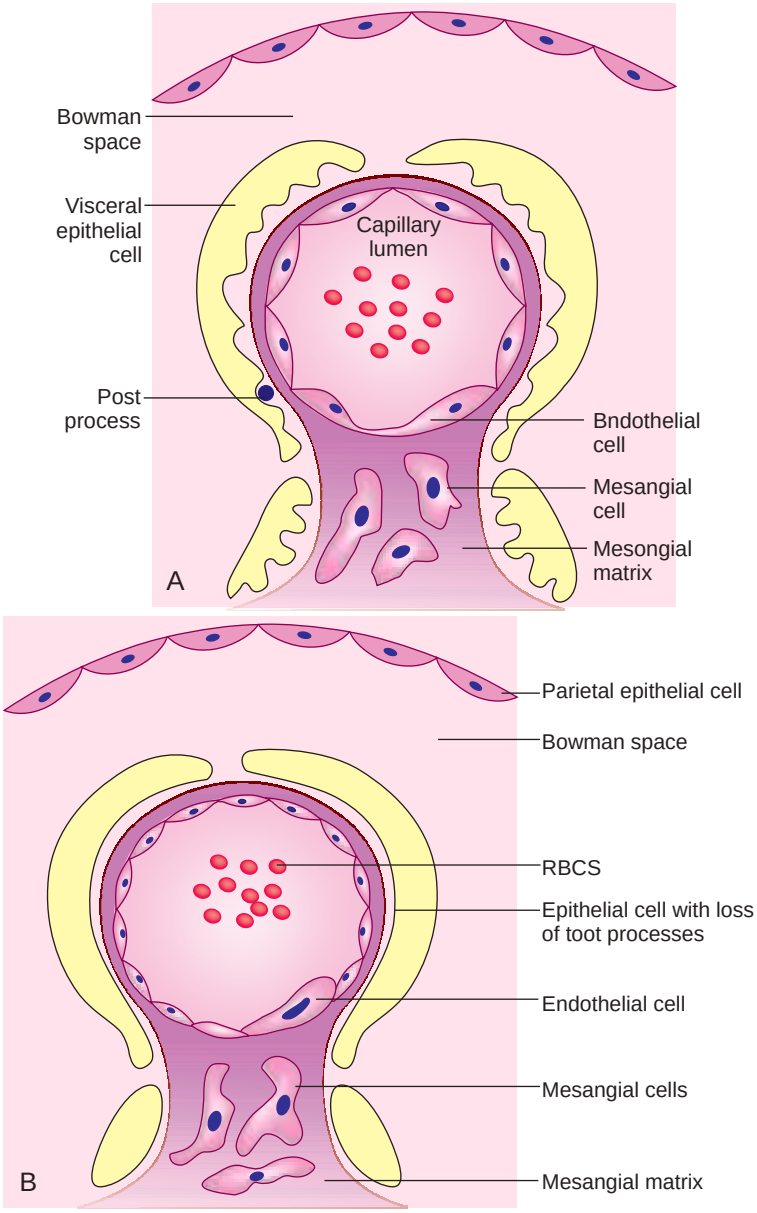


FIGURE 16.7. Diagrammatic representation of electron microscopic appearance of (a) normal glomerulus and (b) glomerulus in MCD showing effacement of foot processes.

No electron-dense deposits are seen. Proximal convoluted tubular cells are lipid laden (therefore, the disease is also called **lipoid nephrosis**).

Focal Segmental Glomerulosclerosis (FSGS)

- Typically shows focal (focal indicates involvement of some glomeruli) and segmental (segmental indicates involvement of part of the glomerulus) sclerosis. It has the following types:
  1. Idiopathic or primary (10–35% patients)
  2. FSGS superimposed on another primary glomerular lesion
  3. Renal ablation FSGS (seen with reflux nephropathy and analgesic abuse)
  4. Secondary FSGS (seen with heroin abuse/HIV/sickle cell disease)
  5. A rare inherited type in which the disease is caused by mutations in genes encoding for glomerular proteins, eg, podocin and α-actinin.
- Eighty percent patients present with nephrotic syndrome.
- Fifty percent convert to end-stage renal disease.

- It differs from minimal change disease in the following ways:
  - Greater incidence of haematuria and hypertension
  - Non-selective proteinuria
  - Poor response to steroids and progression to chronic glomerulonephritis

Pathogenesis

The epithelial damage which is a hallmark of FSGS is caused by different mechanisms:

1. Genetic mechanisms: Genetic defects that affect the integrity of the normal glomerular filtration barrier (eg, mutations in the Nephrosis, Congenital, Finnish Type or NPHS genes, NPHS1 and NPHS2, which encode for the proteins nephrin and podocin, respectively; mutations in the gene encoding the podocyte actin-binding protein α-actinin-4; and mutation in the gene encoding Transient receptor potential calcium channel-6 or TRCP6, a podocyte protein responsible for maintaining calcium flux).
2. Circulating factors: Presence of an unknown circulating factor is thought to be responsible for the epithelial damage as it is noted that the disease recurs even after transplantation.

Pathology

Light microscopy

- Segmental involvement
- Collapse of basement membrane, hyalinosis and lipid droplets in the affected segment, gradually leading to global sclerosis (global means entire glomerulus). The hyalinosis and sclerosis is due to entrapment of plasma proteins (a result of excessive membrane permeability) and increased ECM deposition.
- Unaffected glomeruli show increased mesangial matrix/mesangial proliferation.

Electron microscopy

- Loss of foot processes
- Detachment of epithelial cells and denudation of glomerular basement membrane

Immunofluorescence

IgM and C_3 deposits in sclerotic areas.

Clinical Course

One-fourth patients develop intractable massive proteinuria ending in renal failure within 2 years.

HIV-Associated Nephropathy

- Seen in 5–10% of HIV-infected patients.
- Shows features of severe collapsing FSGS with foci of cystically dilated tubules filled with proteinaceous material. Inflammation and fibrosis may be seen in later stages.
- Electron microscopy shows a large number of tubuloreticular inclusions in endothelial cells. These inclusions are basically interferon α-mediated alterations in the epithelial endoplasmic reticulum.

Membranoproliferative Glomerulonephritis (MPGN)

- As the name suggests MPGN is characterized by proliferation of glomerular cells and changes in the GBM. The proliferation is predominantly mesangial, thus the condition is also called **mesangiocapillary glomerulonephritis.**
- It is responsible for 5–10% cases of idiopathic nephrotic syndrome. It may sometimes arise secondary to SLE, Hepatitis B and C, CLL, α1 AT deficiency, endocarditis, systemic infections, HIV and schistosomiasis.

Clinical Features

- Proteinuria in the nephrotic or non-nephrotic range
- Haematuria

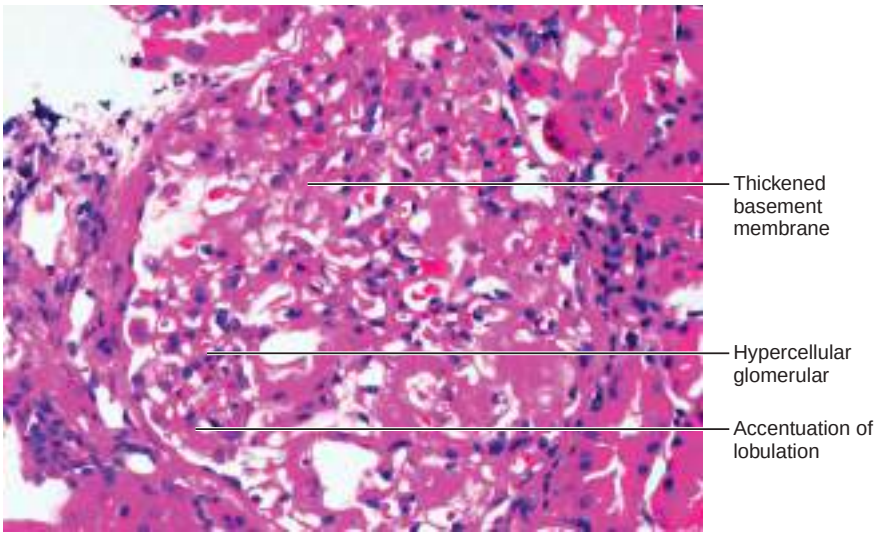


FIGURE 16.8. Microphotograph of membranoproliferative glomerulonephritis showing double contour or tram track appearance (H&E; 400X).

Morphology (Fig. 16.8)

- Large and hypercellular glomeruli showing proliferation of mesangial cells, infiltration by leukocytes and increase in mesangial matrix.
- Also seen is lobular accentuation and formation of epithelial crescents.
- Glomerular basement membrane is thickened and has a **double contour or tram track appearance** due to "duplication" which is formation of a new basement membrane. The new membrane forms consequent to stimulation by the subendothelial deposits of immune complexes. Duplication is followed by inclusion of mesangial, endothelial or leukocytic cells between the two layers leading to splitting of GBM. This change is highlighted with PAS and silver stains).

Types

- *Type I (more common)*:
  - Characterized by subendothelial electron-dense deposits and Clq, C3, C4 and IgG granular deposits.
  - Can be seen with SLE, hepatitis B and C, Schistosomiasis, α-1 AT deficiency, certain malignancies and infected arteriovenous shunts (also called secondary MPGN).
- *Type II*
  - Lamina densa of GBM shows irregular ribbon-like electron-dense deposits of unknown composition **(dense deposit disease).**
  - C3 is present in basement membrane as granular linear deposits and in mesangium as mesangial rings; IgG, C1q and C4 are absent.
  - Excessive complement activation is the fundamental abnormality. MPGN type II. It mainly affects young adults.
  - The patient has decreased serum levels of C3, Factor B and properdin (components of alternative complement pathway) and normal C1q and C4.
  - Normally the alternate pathway C3 convertase is labile. Patients of Type II MPGN have an antibody against C3 convertase called C3 nephritic factor, which binds to C3 convertase and prevents its inactivation, favouring persistent splitting of C3 into C3a and C3b. Mutations in the genes encoding for complement regulatory protein '**Factor H**' facilitate the activation of alternate complement pathway.

IgA Nephropathy (Berger Disease)

- Typically shows prominent IgA deposits in the mesangial region.
- Most common type of glomerulonephritis seen on renal biopsy.

Clinical Features

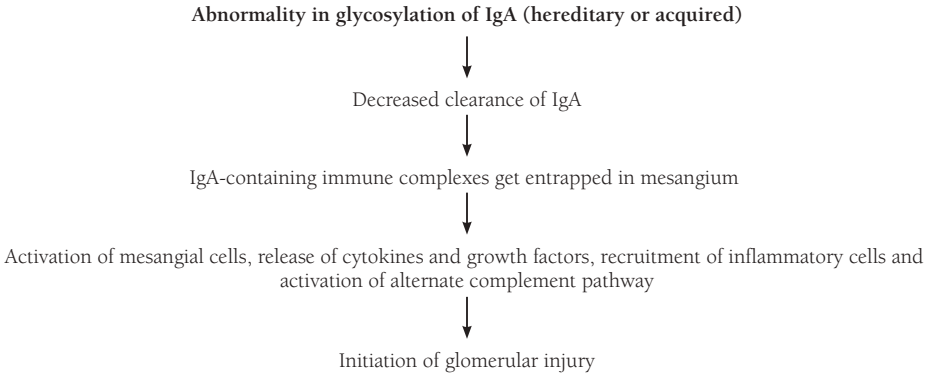
- Affects children and adults.
- Occurs after mucosal (respiratory, gastrointestinal or urinary tract) infections (increased IgA synthesis in response to viruses, bacteria, food allergens, etc.).
- Presents with gross or microscopic haematuria and/or proteinuria.
- Five to ten percent present as acute nephritic syndrome.
- Course of disease variable; many individuals maintain normal renal function for decades.
- Chronic renal failure (CRF) may occur in as many as 50% cases.
- Henoch–Schönlein purpura (a systemic disorder characterized by purpura, abdominal pain and arthritis) has many similarities with IgA nephropathy.

Morphology

- Mesangial widening and segmental inflammation confined to certain glomeruli (focal proliferative GN) or overt crescent formation (crescentic GN) or diffuse mesangial proliferation (mesangioproliferative GN) may be seen.
- Mesangium shows electron-dense deposits.
- IF shows mesangial deposition of IgA, C3, properdin and small amounts of IgG/IgM.

Pathogenesis (Flowchart 16.4)

- Involves abnormality in IgA production and clearance (IgA is the main immunoglobulin in mucosal secretions).



FLOWCHART 16.4. Pathogenesis of IgA nephropathy.

- Normally serum IgA levels are low and it exists predominantly in monomeric form. Polymeric form, which is catabolised by the liver, has a greater tendency of forming immune complexes.
- Plasma polymeric IgA levels are increased in IgA nephropathy
- IgA nephropathy is initiated by either, an increase in production of IgA or formation of circulating IgA-containing immune complexes (due to an abnormality of immune regulation). Increased frequency of IgA nephropathy is noted in celiac disease (characterized by presence of intestinal mucosal defects) and liver disease (characterized by defective hepatobiliary clearance of IgA complexes). Another key factor in the pathogenesis of IgA nephropathy is abnormal glycosylation of IgA due to a hereditary or acquired defects. This abnormally glycosylated IgA may either itself deposit in the glomeruli or initiate an autoimmune response leading to formation of IgG autoantibodies against it. This leads in the formation of circulating immune complexes which deposit in the mesangium.

Q. Differentiate between nephritic and nephrotic syndrome.

Ans. Differences between nephritic and nephrotic syndrome are listed in Table 16.4.

TABLE 16.4. Differences between nephritic and nephrotic syndrome

Features	Nephritic	Nephrotic syndrome
Proteinuria	Usually <1.0 g/day	>3.5 g/day
Haematuria	Present	Absent
Oliguria	Present	Absent
Lipiduria	Absent	Present
Casts	Red cell casts	Lipid casts
Colour of urine	Cocoa coloured/smoky urine	Frothy urine
Azotaemia	Present	Absent
Hyperlipidaemia	Absent	Present
Oedema	Less marked	More marked

Q. Differentiate between membranous glomerulonephritis and minimal change disease.

Ans. Differences between membranous glomerulonephritis and minimal change disease are tabulated in Table 16.5.

TABLE 16.5. Differences between membranous glomerulonephritis and minimal change disease

Features	Membranous glomerulonephritis	Minimal change disease
Age	Adults	Children
Light microscopy	Thickening of GBM (GBM width in healthy adults is 300–400 nm)	Normal GBM
Electron microscopy	Granular subepithelial deposits	Foot process effacement and lipid-laden cells in PCT
Immunofluorescence	Granular deposits of IgG and C3	No deposition
Hypertension	Present	Absent
Haematuria	Present	Absent
Corticosteroid therapy	Minimal response	Good response

Q. Write briefly on the aetiopathogenesis, clinical features and pathology of tubulointerstitial nephritis (TIN).

Ans. TIN is defined as inflammation of the tubules and interstitium with sparing of the glomeruli or their involvement in very late stages. It has two components:

- **Pyelonephritis** (usually due to bacterial infections) is a term applied to TIN with prominent involvement of renal pelvis in addition to tubules and interstitium.
- The term **interstitial nephritis** is reserved for cases of TIN that are nonbacterial in origin (include tubular injury due to drugs, metabolic disorders, physical and immunologic injury).

Pyelonephritis

Pathogenesis

Principal causative organisms:

Enteric Gram-negative rods, mainly *Escherichia coli*, *Proteus*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Staphylococcus* and *Streptococcus faecalis*. Rarely mycobacterial, fungal and viral organisms.

Predisposing conditions:

- Urinary tract manipulations, eg, catheterization, urethral instrumentation and cystoscopy
- Congenital or acquired anomalies of the urinary tract (intrarenal reflux, vesicoureteral reflux and deranged vesicoureteric junction)
- Outflow obstruction (nodular hyperplasia prostate, uterine prolapse, calculi, strictures, tumours and neurogenic bladder)
- Immunodeficiency or immunosuppression

Routes of spread of bacteria to kidneys:

1. Haematogenous: Seen in septicaemia or infective endocarditis and is less common.
2. Ascending infection: This is more common and occurs by the following mechanism (Flowchart 16.5):

Attachment of bacteria to the urothelial lining by fimbriae
↓ *Facilitated by genetically determined properties of the urothelium and bacterial pathogens*
Colonization of distal urethra (introitus in females)
↓
Access of bacteria into the bladder due to catheterization, urethral instrumentation and cystoscopy

FLOWCHART 16.5. Spread of bacteria to kidneys by ascending infection.

- In the absence of instrumentation, UTI more commonly affects females because of the **proximity of urethra to rectum (colonization by enteric bacteria favoured).**
- Other factors aiding to the development of UTI in women are presence of **a short urethra, trauma to the urethra during sexual intercourse** and **pregnancy**.
- **Incompetent vesicoureteric orifice** in children allows bacteria to ascend the ureters. Normally the ureters are inserted into the bladder in a way that prevents retrograde flow of urine into the ureters, especially during micturition when the intravesical pressure rises. Incompetency of the opening allows retrograde flow of urine into the ureters and this is called vesicoureteral reflux (VUR). This is present in 20–40% of children with UTI.
- **Intrarenal reflux** is a condition in which the infected bladder urine is propelled into the renal pelvis and into the renal parenchyma through the open ducts at the tips of the renal papillae.

Types

1. **Acute pyelonephritis**
   (a) Urinary tract infection may involve the upper urinary tract (pyelonephritis) or the lower urinary tract (cystitis, prostatitis and urethritis).
   (b) Infections of the lower urinary tract may remain localized or may spread to involve the kidney.
   (c) Acute suppurative inflammation of the renal tubules and interstitium is called acute pyelonephritis.

***Gross Morphology*:**
- Affects one or both kidneys.
- Affected kidney is normal in size or slightly enlarged.
- Discrete yellow, raised abscesses are seen on the renal surface.

***Microscopy*:**
- Necrosis and abscess formation in the renal parenchyma.
- Abscesses limited to the interstitium initially, moving into the tubules later.
- Large masses of neutrophils in the tubules give rise to the characteristic WBC casts.
- When obstruction is severe, it prevents the drainage of pus leading to pus filling up the renal pelvis, calyces and ureters **(pyonephrosis)**.
- **Papillary necrosis** is a relatively rare form of pyelonephritis in which there is necrosis of the tips of the renal papillae (particularly common in diabetes and analgesic abuse). Development of papillary necrosis is associated with a poor prognosis.
- The pathognomonic morphological finding of papillary necrosis is a sharply defined area of yellow necrosis in the apical two-thirds of the renal pyramid.

***Clinical features*:**
- Sudden onset of pain at the costovertebral angle, fever, chills and malaise.
- Signs of bladder irritation like dysuria, frequency and urgency.
- Urine examination shows pyuria and bacteriuria (culture shows growth).

- Usually self-limiting; in the presence of predisposing conditions may become recurrent or chronic.

2. **Chronic pyelonephritis (CPN) and reflux nephropathy**
   Morphological entity in which interstitial inflammation and scarring of renal parenchyma is associated with scarring and deformity of the pelvocalyceal system.

***Types*:**

(a) Chronic obstructive pyelonephritis
   - Recurrent infections occurring in a background of obstruction which lead to repeated inflammation and scarring.
   - The disease can be bilateral as in congenital anomalies of the urethra (posterior urethral valves) or unilateral as in calculi and unilateral obstructive lesions.

(b) Chronic reflux–associated pyelonephritis
   This more common form of CPN results from the superimposition of UTI on congenital vesicoureteral reflux and intrarenal reflux. May be unilateral or bilateral.

***Gross Morphology*:**

- May be unilateral or bilateral, patchy or diffuse.
- Coarse, discrete corticomedullary scars are seen corresponding to the overlying blunted or dilated calyces.
- Asymmetrical pelvocalyceal scarring leads to blunting of papillae and deformity of calyces.

Microscopy (Fig. 16.9):

- Uneven interstitial fibrosis with interstitial inflammatory infiltrate composed of lymphocytes, plasma cells and rarely neutrophils.
- Dilatation as well as contraction of tubules showing atrophy of lining epithelium.
- Dilated tubules contain pink PAS-positive casts called 'colloid casts' that resemble colloid in thyroid (thyroidization).
- Fibrosis of calyceal mucosa.
- Vascular changes similar to benign arteriosclerosis.
- Late stages may show glomerulosclerosis secondary to nephron loss.

***Clinical features*:**

- Presents as gradual onset of renal insufficiency (azotaemia); often noticed due to hypertension.
- Ultrasonography is used to determine the size of the kidney and a pyelogram is used to show the asymmetrical contraction of kidneys, blunting and deformity of the pelvocalyceal system.

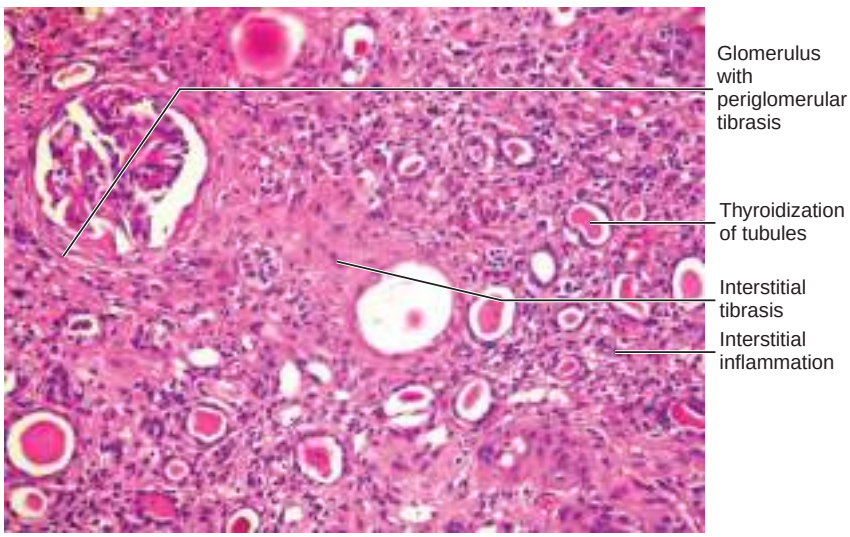


FIGURE 16.9. H&E-stained section from kidney showing uneven interstitial fibrosis with an inflammatory infiltrate and dilatation and contraction of tubules with atrophy of lining epithelium. Dilated tubules contain pink PAS-positive casts.

Q. Differentiate between acute and chronic pyelonephritis.

Ans. Differences between acute and chronic pyelonephritis are listed in Table 16.6.

TABLE 16.6. Differences between acute and chronic pyelonephritis

Features	Acute pyelonephritis	Chronic pyelonephritis
Definition	Acute suppurative inflammation of kidney caused by bacterial infection	Chronic tubulointerstitial disease showing tubular inflammation and renal scarring with pathologic involvement of calyces and pelvis
Predisposing factors	Urinary obstruction, instrumentation, catheterization, pregnancy, DM and pre-existing renal lesions	Obstructive pathology, presence of reflux
Subtypes	No subtypes	Chronic obstructive pyelonephritis and reflux-associated nephropathy
Gross	Discrete yellow, raised abscesses are seen on the renal surface	Coarse corticomedullary scars overlying blunted or dilated calyces
Microscopy	Tubules show suppurative necrosis with preserved outline	• Tubules show atrophy in some areas and dilatation in others • Dilated tubules filled with colloid-like casts (thyroidization)
Clinical features	Sudden onset with back pain, fever and malaise	Insidious (silent) onset with progressive decline in renal function

Q. Differentiate between chronic glomerulonephritis (CGN) and chronic pyelonephritis (CPN).

Ans. Contrasting features of CGN and CPN are given in Table 16.7.

TABLE 16.7. Contrasting features of CGN and CPN

Features	CGN	CPN
Primary disease	Glomerular, cortical	Tubulointerstitial
Distribution	Diffuse	Patchy
Proteinuria	Marked	Mild or absent
Involvement	Symmetrical	Asymmetrical
Cortical scars	Fine	Coarse
Calyces	Normal	Distorted
Glomerulosclerosis	>50%	Occasional
Interstitial fibrosis	Mild	Marked
Periglomerular fibrosis	Mild	Marked
Tubular atrophy or loss	Mild	Marked
Thyroidization	Absent	Present

Q. Describe the Clinico morphological features and pathogenesis of diabetic nephropathy.

Ans. Morphological Changes in Diabetic Glomerulosclerosis

Clinical Features of Diabetic Glomerulosclerosis

Patients may present with either of the three glomerular syndromes, namely, non-nephrotic proteinuria, nephritic syndrome and chronic renal failure.

1. **Capillary basement membrane thickening:**
   Widespread thickening of GBM with mesangial widening

2. **Diffuse glomerulosclerosis:**
   (a) Overall thickening of GBM
   (b) Diffuse increase in mesangial matrix with proliferation of mesangial cells
   (c) Exudative lesions
      (i) Capsular hyaline drops (eosinophilic hyaline thickening of the parietal layer of Bowman's capsule, which bulges into glomerular space).
      (ii) Fibrin caps (homogenous, brightly eosinophilic material in the wall of a peripheral capillary of a lobule).
3. **Nodular glomerulosclerosis (Kimmelstiel–Wilson lesions/intercapillary glomerulosclerosis):**
   (a) These lesions are specific for juvenile onset DM or islet cell antibodies-positive DM.
   (b) One or more nodules are seen in glomeruli accompanied by thickening of basement membrane of surrounding capillaries.
   (c) Nodules are ovoid, spherical, laminated, hyaline, acellular and PAS-positive masses, which contain lipid and fibrin and compress capillaries to obliterate glomerular tufts leading to tubular atrophy and interstitial fibrosis.
4. **Vascular lesions**
   (a) Atheromas in renal arteries.
   (b) Hyaline arteriosclerosis of afferent and efferent arterioles.
   (c) These vascular lesions are responsible for renal ischaemia, which results in tubular atrophy and interstitial fibrosis.
   (d) The above-mentioned changes may result in a small contracted kidney.
5. **Diabetic pyelonephritis:** Poorly controlled diabetics are susceptible to bacterial infection and acute pyelonephritis. Papillary necrosis is an important complication.
6. **Tubular lesions (Armanni–Ebstein lesions):** In untreated diabetics, who have high blood sugar levels, the epithelial cells of PCT develop extensive glycogen deposits appearing as vacuoles.

Pathogenesis of Diabetic Glomerulosclerosis

- **Metabolic defects:** Insulin deficiency and recurrent hyperglycaemia.
- **Biochemical changes in GBM:** Increased collagen and fibronectin, decreased proteoglycans and heparin sulphate.
- **Nonenzymatic glycosylation** of haemoglobin and other proteins (collagen and BM material), resulting in thickening of BM.
- **Haemodynamic changes:** ↑ GFR associated with glomerular hypertrophy.

Q. Describe the aetiopathogenesis, clinical features and morphology of acute tubular injury (ATI) or acute kidney injury (AKI).

Ans. ATI is a reversible disorder characterized by destruction of tubular epithelial cells and acute suppression of renal function. It is the most common cause of acute renal failure. Other causes of acute renal failure besides ATI include

- Severe glomerular disease, eg, RPGN
- Diffuse renal vascular disease, eg, microscopic polyangiitis and thrombotic microangiopathies
- Acute papillary necrosis associated with acute pyelonephritis
- Acute drug-induced interstitial nephritis
- Urinary obstruction due to tumours, NHP, blood clots, etc.

Types

1. Ischaemic ATI
2. Nephrotoxic ATI

Pathogenesis

Tubular epithelial cells are particularly sensitive to anoxia and toxins. There are two important causes of ATI:

1. Direct tubular injury
2. Ischaemia

In **ischaemic ATI**, ischaemia leads to vasoconstriction induced by renin–angiotensin system; whereas, in **toxic ATI** there is direct damage to tubules. Tubular cell injury is followed by the following sequence of events:

- Desquamation and detachment of tubular epithelial cells
- Tubular obstruction by oedema, desquamated cells and casts
- Increased intratubular pressure and decreased tubular flow
- Tubular rupture and back-leak of tubular fluid into interstium
- Increased interstitial pressure and compression of tubules and blood vessels causing further ischaemia and reduced GFR leading to oliguria

Morphology

Ischaemic ATI is characterized by necrosis of short segments of tubules.

- Most lesions are seen in the straight portion of proximal tubule and ascending thick loop of Henle.
- There is blebbing of brush border, vacuolization of cells, detachment of tubular cells from their basement membrane and sloughing in the urine.
- Proteinaceous casts are present in distal tubules and collecting ducts; these casts consist of Tamm–Horsfall protein, secreted normally by tubular epithelial cells along with haemoglobin and other plasma proteins. In the later stages, disruption of tubular basement membrane (tubulorrhexis) adjacent to the casts may be seen.
- The interstium shows oedema and inflammatory infiltrate.

Toxic ATI demonstrates a similar morphology, but tubular necrosis is most prominent in proximal tubules and tubular basement membrane is spared. The appearance varies depending on the cause of toxic ATI.

Epithelial regeneration is seen in the form of mitotic activity and replacement of tubular lining by cuboidal cells.

Clinical Course

ATI evolves through three stages:

1. Initial: Lasts for about 36 h; is dominated by the signs and symptoms of the causative event; there is an increase in BUN due to a transient decrease in renal output.
2. Maintenance: During the maintenance phase, renal tubule injury is established, the GFR stabilizes at a level well below normal and the urine output is low or absent. Although oliguria (or anuria) is one of the clinical hallmarks of ATI, it is absent in a minority of patients (ARF due to nephrotoxins is typically nonoliguric). The second phase of ATI lasts usually for 1–2 weeks but may extend to a few months.
3. Recovery: The recovery phase of AKI is characterized by polyuria and gradual normalization of GFR; however, when there is multiorgan dysfunction, regeneration of renal tissue may be severely impaired, and renal function may not return.

Q. Differentiate between ischaemic and nephrotoxic ATI.

Ans. Differences between ischaemic and nephrotoxic ATI are listed in Table 16.8.

TABLE 16.8. Differences between ischaemic and nephrotoxic ATI

Features	Ischaemic AKI	Nephrotoxic AKI
Definition	ATI caused by renal ischaemia	ATI caused by toxic agents
Causes	Shock, mismatched blood transfusion, haemolytic crises, myoglobinuria, acute pancreatitis and septicaemia	Nephrotoxins like heavy metals, eg, mercuric organic solvents, gentamycin and amphotericin B, cisplatin and radiographic contrast media
Distribution of lesions	Straight portion of proximal tubule and ascending thick loop of Henle	Proximal convoluted tubules
Pathology	Blebbing and sloughing of brush border, detachment of tubular cells from their basement membrane and their sloughing in the urine	Tubular basement membrane is spared. Mercury salts cause coagulative necrosis, CCL4 causes lipoid degeneration and ethylene glycol causes hydropic degeneration of the PCT
Oliguria	Present	Typically nonoliguric
Casts	Eosinophilic and pigmented granular casts consisting of Tamm–Horsfall protein, haemoglobin, myoglobin and other plasma proteins are present	Nonspecific; dependent on the causative agents, eg, lipid casts are present in CCl4 poisoning

Q. Differentiate between benign and malignant nephrosclerosis.

Ans. Differences between benign and malignant nephrosclerosis are listed in Table 16.9.

TABLE 16.9. Differences between benign and malignant nephrosclerosis

Features	Benign nephrosclerosis	Malignant nephrosclerosis
Cause	Benign hypertension, DM, increasing age	Malignant hypertension
Gross	Leather grain appearance	Flea-bitten appearance due to tiny petechial haemorrhages
Microscopy	• Narrowing of the lumen of arterioles caused by thickening and hyalinization of the walls (hyaline arteriosclerosis) • Fibroelastic hyperplasia of arteries and arterioles	• Hyperplastic arteriolitis (onion-skinning) due to proliferation and elongation of smooth muscle cells • Necrotizing glomerulitis (neutrophilic infiltration and thrombosis of capillaries) • Fibrinoid necrosis of arterioles (necrotizing arteriolitis)
Clinical features	• Hypertension • Microscopic haematuria • Contracted kidney • Trace proteinuria	• Accelerated hypertension with renal impairment, encephalopathy and retinopathy • Enlarged kidneys • Marked proteinuria

Q. Describe the aetiopathogenesis, gross appearance and complications of renal calculi/urolithiasis.

Ans. The clinicopathological features of various renal calculi/urolithiasis are summarized in Table 16.10.

TABLE 16.10. Clinicopathological features of various renal calculi/stones

Type of calculi	Incidence	Causes	Pathogenesis	Gross
Calcium stones	75–80%	• Idiopathic • Hypercalciuria • Hypercalcaemia • Hyperoxaluria • Hyperuricosuria • Primary hyperthyroidism • Distal renal tubular acidosis	Super saturation of calcium ions in urine, alkaline pH of urine	Small, smooth contour, or irregular jagged mass of spicules
Struvite stones [$MgNH_4(PO)_3$] triple stone/ stag-horn stone	10–15%	Urinary infection by urease-containing organisms like *Proteus*	Alkaline urinary pH due to production of ammonia from urea (by urease)	Large, solitary, branching structure formed due to progressive accretion of salts
Uric acid stones	6%	Gout, dehydration, idiopathic and malignant tumours	Acidic urine and ↓ solubility of uric acid	Smooth, yellow to brownish, hard and multiple
Cystine stones	1–2%	Hereditary	Cystine precipitates in acidic urine	Small, smooth yellow, multiple and round
Others	Up to 10%	Inherited abnormality of amino acid metabolism	Xanthinuria	

Complications of Urolithiasis

1. Loss of function in the affected kidney
2. Obstruction of the ureter (acute unilateral obstructive uropathy) and hydronephrosis; secondary infection gives rise to pyonephrosis
3. Urinary tract infection
4. Haematuria

Q. Classify renal tumours and describe the clinicopathological features of renal cell carcinoma (RCC).

Ans. See Table 16.11 for classification of renal tumours.

TABLE 16.11. Classification of renal tumours

Origin	Benign	Malignant
Epithelial tumours of renal parenchyma	Adenoma, oncocytoma, adrenal rests	Renal cell carcinoma (RCC or hypernephroma)
Epithelial tumours of renal pelvis	Transitional cell papilloma	Transitional cell carcinoma (TCC), squamous cell carcinoma, adenocarcinoma of renal pelvis
Embryonal tumours	Mesoblastic nephroma, multicystic nephroma	Wilms tumour
Nonepithelial tumours	Angiomyolipoma, fibroma, leiomyoma	Sarcoma
Miscellaneous	Reninoma	–
Metastatic tumours	–	–

RCC

- Age: > 60 years
- Male:female ratio = 2:1 to 3:1
- Constitutes up to 90% of all primary malignant tumours of the kidney, 2–3% of all cancers.

- Also called 'hypernephroma' due to resemblance to clear cells of adrenal cortex and gross yellow colour
- Arises from tubular epithelium (renal adenocarcinomas)

Epidemiology

- Predisposing factors: Smoking, obesity, hypertension, unopposed oestrogen therapy, exposure to asbestos, cadmium, petroleum products and heavy metals and acquired cystic disease in patients with long-standing dialysis.
- Majority of cases of RCC are sporadic; about 5% are inherited and associated with:
  1. von Hippel–Lindau (VHL) syndrome: Predisposition to a large number of neoplasms, mainly haemangioblastomas of cerebellum and retina, multiple bilateral renal cysts, pheochromocytomas and multicentric bilateral renal cell carcinomas.
  2. Hereditary leiomyomatosis and renal cell cancer syndrome: Autosomal dominant inheritance; mutation in Fumarate Hydratase (FH) gene; associated with uterine and cutaneous leiomyomas and an aggressive variety of papillary RCC.
  3. Hereditary papillary RCC: Autosomal dominant inheritance; multiple cytogenetic abnormalities; mutation in MET proto-oncogene; associated with multiple bilateral papillary RCCs.
  4. Birt–Hogg–Dube (BHD) syndrome: Autosomal dominant inheritance; mutation in BHD gene (expresses folliculin); associated with skin appendageal tumours of hair follicular origin, pulmonary cysts and renal tumours.

Gross Morphology

- Globular, encapsulated, 3–5 cm, soft, lobulated with a variegated appearance (grey-white to yellow with necrosis, haemorrhage and cyst formation); invades or grows into pelvis.
- Polar in distribution; the upper pole is more commonly involved than the lower pole.
- Renal vascular invasion is common.
- Usually sharply defined; however, small satellite nodules are often found in the surrounding substance.
- Enlarges → bulges into pelvis and calyces → fungates through walls of collecting system → ureters.
- Penetrates through capsule → invades perinephric fat and adrenals.

The clinicopathological features of the most common types of RCC are described in Table 16.12.

TABLE 16.12. Clinicopathological features of the most common types of RCC

Features	Clear-cell RCC	Papillary RCC	Chromophobe RCC	Collecting duct (Bellini duct) carcinoma	Xp11 translocation carcinoma
Incidence	70–80%	10–15%	5–8%	1%	
Genetics	• Majority sporadic. • 98% show loss of material on the short arm of chromosome 3 • Second allele lost by somatic mutation • Loss of both copies of VHL gene gives rise to clear-cell carcinoma	• Culprit (tyrosine kinase receptor for the hepatocytic growth factor) is on chromosome 7q31 • Duplication of chromosome 7 increases gene dosage of MET oncogene leading to abnormal growth of distal tubular cells	• Multiple chromosomal loss and hypodiploidy • Arise from intercalated cells lining collecting ducts	• Several chromosomal abnormalities seen but no definite pattern recognized	• Seen in young patients and is associated with overexpression of TFE3 transcription factor due to translocations of TFE3 gene located at Xp11.2 with a number of other genes.

Continued

TABLE 16.12. Clinicopathological features of the most common types of RCC—cont'd

Features	Clear-cell RCC	Papillary RCC	Chromophobe RCC	Collecting duct (Bellini duct) carcinoma	Xp11 translocation carcinoma
Gross	• Solitary, unilateral, bright yellow to grey-white with prominent cystic change and haemorrhage • Aggressive; may infiltrate into surrounding substance, collecting system, calyces, ureters and renal vein	Multifocal, bilateral, less yellow due to lower lipid content, papillae may be seen, haemorrhagic and cystic areas present	• Tan brown • Excellent prognosis	Seen in medullary region	-
Microscopy	Solid to tubular growth pattern, round cells with clear (due to glycogen and lipid) or granular cytoplasm (Fig. 16.10); may show nuclear atypia and giant cells	Papillae lined by, cuboidal to low columnar cells; psammoma bodies present	Solid sheets of cells arranged around blood vessels, individual cell is eosinophilic with well-defined cytoplasmic margins and perinuclear halo	Irregular channels lined by malignant cells with a hobnail appearance; cells enmeshed within a fibrotic stroma	Clear cytoplasm with papillary architecture

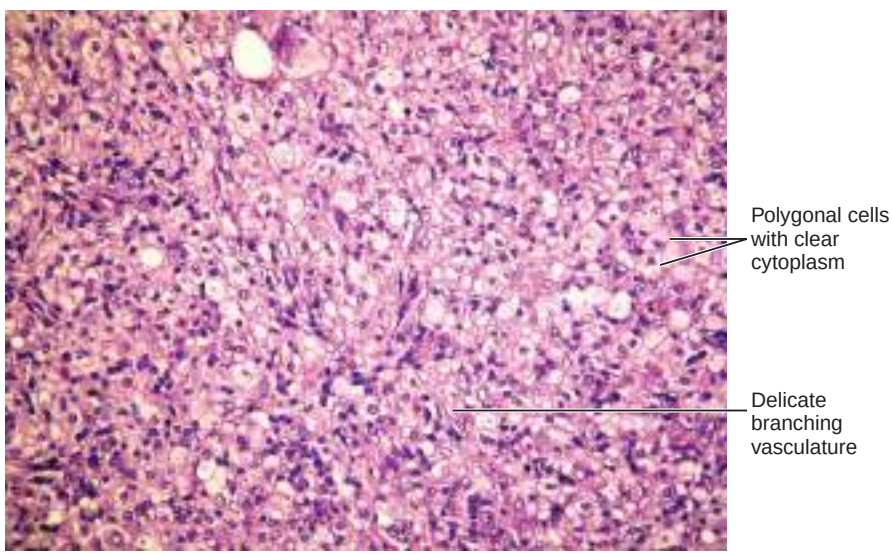


FIGURE 16.10. H&E-stained section from a clear-cell RCC showing clear cells separated by a fine fibrovascular stroma.

Clinical Features

- The three classic diagnostic clinical features of RCC are **painless intermittent haematuria, palpable abdominal mass** and **costovertebral pain** but they are rarely seen together. Most common presentation is intermittent haematuria.
- Fever and constitutional symptoms are commonly seen.

- RCC produces a number of paraneoplastic syndromes due to abnormal hormone production including **polycythaemia** due to elaboration of erythropoietin, **hyperkalaemia, hypertension, Cushing syndrome, leukaemoid reactions, amyloidosis, feminization and masculinization**.
- Tendency to invade renal vein and growth as a solid column even up to inferior vena cava and right side of heart may be seen.
- RCC has a tendency for early and wide spread metastases before giving rise to any local signs and symptoms. Most common sites are lungs and bones.

Prognosis

Five-year survival rate is about 45–70% in the absence of distant metastasis.

Q. Write briefly on Wilms tumour.

Ans. Wilms tumour/nephroblastoma is the most common primary renal tumour of childhood. It has a peak age of 2–5 years and a sex ratio of 1:1. It is associated with three syndromes:

1. WAGR syndrome, characterized by:
   (a) Aniridia
   (b) Genital anomalies
   (c) Mental retardation
   (d) Germline WT1 deletion followed by a nonsense or frame shift mutation of second WT-1 allele (WT-1 gene is present at 11p13 and its protein product is a transcriptional factor).
2. Denys–Drash syndrome, characterized by:
   (a) Gonadal dysgenesis (male pseudohermaphroditism)
   (b) Renal abnormalities (diffuse mesangial sclerosis leading to renal failure)
   (c) Missense mutation of WT-1 affecting DNA-binding properties
3. Beckwith–Wiedemann syndrome, characterized by:
   (a) Enlargement of the body organs (organomegaly)
   (b) Hemihypertrophy, macroglossia, omphalocele, renal medullary cysts and abnormal large cells in adrenal cortex (adrenocytomegaly)
   (c) WT-2 abnormalities (WT-2 gene is present on 11p15.5; its function is unknown; however, WT-2 mutation is known to increase the risk of Wilms tumour).

Morphology

***Gross*:** Large, solitary, well-circumscribed mass, rarely bilateral or multicentric; soft, homogeneous, tan to grey in colour; foci of haemorrhage and necrosis may be present.

Microscopic Features

- Recapitulates different stages of nephrogenesis. Typically shows a classic triphasic combination of blastema (sheets of small blue cells), epithelial elements abortive tubules or glomeruli) and stroma (fibrocystic or myxoid in nature).
- Rarely heterologous elements are identified including squamous or mucinous epithelium, smooth muscle cells, adipose tissue, cartilage, osteoid and neurogenic tissue.

Clinical Features

Palpable abdominal mass, haematuria, pain and hypertension

Metastasis

- Through blood to lung and liver
- Renal (hilar) and paraaortic lymph nodes

Prognosis

Five-year survival rate is above 75%.

Q. Differentiate between RCC and Wilms tumour.

Ans. Differences between RCC and Wilms tumour are listed in Table 16.13.

TABLE 16.13. Differences between RCC and Wilms tumour

Features	RCC	Wilms tumour
Age	Adults	Children
Associated genes	VHL, MET	WT-1 and -2
Gross	• Polar distribution	• Large, rapidly growing mass, which overwhelms the kidney and can replace it entirely
	• Variegated appearance	• Homogeneous appearance
	• Bright yellow to grey-white with prominent cystic change and haemorrhage	• Tan to grey • Haemorrhage and cystic change occasionally seen
Microscopy	Solid to tubular growth pattern, round cells with clear or granular cytoplasm (glycogen and lipid); may show papillae (papillary variant), nuclear atypia and giant cells	Classic triphasic combination of blastemal (sheets of small blue cells), epithelial cells (arranged as abortive tubules or glomeruli) and stromal cells (fibrocystic or myxoid in nature)
Paraneoplastic syndromes	Very common	Usually not seen
Tendency to invade renal vein	Common	Usually not seen
Prognosis	Comparatively poor	Better

Q. Enumerate the causes of a small contracted kidney.

Ans. Causes of a small contracted kidney:

1. Nephrosclerosis: Symmetrically atrophic kidneys with fine, pale, granularity (resembles grain leather)
2. CGN: Symmetrically contracted kidneys with red brown, diffusely granular surface, corticomedullary junction (CMJ) not well made out
3. CPN: One or both kidneys may be involved (asymmetric, diffuse or patchy involvement), coarse scars, poorly defined CMJ, thickening of pelvic mucosa with yellow tinge and pelvocalyceal deformities
4. Late stages of diabetic nephropathy
5. Late stages of amyloidosis
6. Multiple myeloma
7. Gout
8. Senile nephritic syndrome

A contracted kidney with large scars is most commonly the result of:

1. Old infarcts
2. Polyarteritis nodosa

Q. Enumerate the causes of a large white kidney.

Ans. Causes of a large white kidney (pale, soft and grey kidney which weighs more than 250 g) are:

- Acute diffuse GN and RPGN
- Lipoid nephrosis
- Early DM and amyloidosis
- SLE
- Toxaemia
- Leukaemia
- Malaria
- Irradiation nephritis/chemotherapy

Q. Write briefly on urothelial (transitional cell) tumours.

Ans. Tumours of urinary bladder include

1. Urothelial (transitional cell) tumours
   (a) Exophytic papilloma
   (b) Inverted papilloma
   (c) Papillary urothelial neoplasms of low malignant potential
   (d) Low-grade and high-grade papillary urothelial cancers
   (e) Carcinoma in situ (CIS or flat non-invasive urothelial carcinoma)
2. Mixed carcinoma
3. Adenocarcinoma
4. Small cell carcinoma
5. Sarcomas

WHO grading of urothelial (transitional cell) tumours is given in Table 16.14.

TABLE 16.14. Grading of urothelial (transitional cell) tumours of the urinary bladder

WHO grading
Urothelial papilloma
Papillary urothelial neoplasms of low malignant potential
Low- or high-grade papillary urothelial cancers
Carcinoma in situ (CIS or flat non-invasive urothelial cancers

Peak Age

50–80 years

Gender Distribution

Male:female ratio = 3:1

Pathogenesis

Tumours arising from urothelium are known to be associated with the following:

- Cigarette smoking
- Industrial exposure to aryl amines
- *S. haematobium* infection
- Long-term use of analgesics
- Long-term exposure to cyclophosphamide
- Monosomy of chromosome 9
- Deletions of 9p and 9q as well as deletions of 17p, 13q, 11p and 14q
- 9p deletions (9p21) involve the tumour-suppressor gene p16 (MTS 1 and INK 4 alfa, which encodes an inhibitor of a cyclin-dependent kinase and also the related p15

Morphology

- Exophytic papillomas are small pedunculated lesions composed of a connective tissue stalk covered by normal appearing urothelium.
- Inverted papillomas show bland appearing epithelium extending down into the lamina propria.
- Papillary urothelial neoplasms of low malignant potential differ from a papilloma in having a thicker urothelial layer.
- Low-grade papillary urothelial carcinomas show mild atypia and increased mitoses but have an orderly architecture and maintain nuclear polarity. They may infrequently recur or rarely invade.

- High-grade papillary urothelial carcinomas show marked atypia and increased mitoses including atypical ones. They have a disorderly architecture and show loss of nuclear polarity and dyscohesiveness. They have major potential for recurrence and metastases.

Clinical Features

Painless haematuria, frequency, urgency and dysuria

Complications

Stricture formation, hydronephrosis and pyonephrosis

Prognosis

Depends on tumour grade or blood group antigens (tumour cells expressing A, B and H antigens have a better prognosis)

17

Male Genital Tract

TESTIS AND EPIDIDYMIS

Normal Structure

- The **scrotal sac** lodges the **testis** and the **epididymis** along with the **lower part of spermatic cord.**
- The testes are a pair of ovoid glandular structures that are responsible for the production of sperms and the male sex hormone testosterone.
- They are invaginated by the **tunica** which has three layers, namely, the **tunica vasculosa, albuginea** and **vaginalis**. **Tunica vasculosa** is the innermost connective tissue layer of the tunica which carries blood vessels to the testis. It is covered by the **tunica albuginea** which encases the testis and also extends into it. Overlying this is the outer layer of the tunica, the **tunica vaginalis.**
- Each testis is divided by invaginations of the tunica albuginea into small compartments called **lobules**. Each lobule contains numerous tightly coiled **seminiferous tubules**, the walls of which contain the **germ cells, Sertoli cells** and **Leydig cells**.
- The germ cells multiply and differentiate to produce **spermatocytes** from the onset of puberty. The spermatocytes develop into **spermatids** and eventually **spermatozoa**. About 400 million sperms are released in a single ejaculation. **Sertoli cells** provide support to the developing sperm cells. The seminiferous tubules are held together by loose connective tissue called **interstitium** which lodges the Leydig cells. **Leydig cells** produce testosterone that is responsible for the secondary sex characteristics associated with males.
- The tubules become less convoluted towards the lobular apex and continue as 20–30 straight **collecting ducts**. These ducts merge to form the **rete testis** lined by flattened epithelium. The secretions from rete testis drain into the **vasa efferentia** which opens at the upper pole of the epididymis. The lower pole of the epididymis merges with the **ductus deferens.**

Q. Write briefly on cryptorchidism.

Ans. Cryptorchidism is derived from the Greek words *kryptos*, meaning hidden and *orchis*, meaning testicle and indicates the absence of one or both testes from the scrotum. It has the following salient features:

- It is usually seen at birth but can rarely develop later in life. About 80% of cryptorchid testes descend by the first year of life making the actual incidence about 0.8%.
- The exact cause is not known but the risk factors include intrauterine growth retardation, prematurity, perinatal asphyxia, C-section and toxaemia of pregnancy.
- An undescended testis can be found anywhere along the 'normal path of descent' from the retroperitoneum (25% cases) to the inguinal ring (70% cases) or any other location in the inguinal canal (5% cases).
- It is sometimes found outside the normal pathway, eg, the thigh, the perineum, the opposite scrotum or the femoral canal, when it is labelled *ectopic or wandering testis*. An underdeveloped undescended testis is labelled *hypoplastic*.
- In rare cases, the testis appears to have vanished (true hidden testes or 'anorchia').

- An undescended testis is called *retractile* when it can be manipulated into scrotum where it remains without tension. On the other hand, when it can be manipulated into upper scrotum but retracts when released, it is called *gliding*.
- Cryptorchidism is unilateral in about 80% cases and bilateral in the remaining. Most cases are clinically asymptomatic and discovered only on physical examination.
- Cryptorchid testes can be brought into the scrotum by a surgical procedure called an 'orchiopexy'.
- Untreated cases may be associated with reduced fertility, increased risk of testicular germ cell tumours and are also more prone to torsion, infarction and inguinal hernia.
- On gross examination, the cryptorchid testis is small and fibrotic. Histologically, there is marked reduction in the number of germ cells.

Q. Write briefly on testicular atrophy.

Ans. Testicular atrophy is a regressive change which can have a varied aetiology.

Causes

- Progressive atherosclerotic narrowing of testicular blood vessels, as in old age
- End stage of all inflammatory conditions (orchitis)
- Cryptorchidism
- Hypopituitarism
- Obstruction of flow to semen
- Malnutrition and cachexia
- Prolonged administration of female sex hormones
- Exhaustion atrophy due to high level of pituitary follicle-stimulating hormone
- Klinefelter syndrome

Gross Morphology

Testes are small in size and firm in consistency due to fibrotic changes.

Microscopy (Fig. 17.1)

- Spermatic tubules show hyalinization and thickening of basement membrane.
- There is increased interstitial connective tissue.

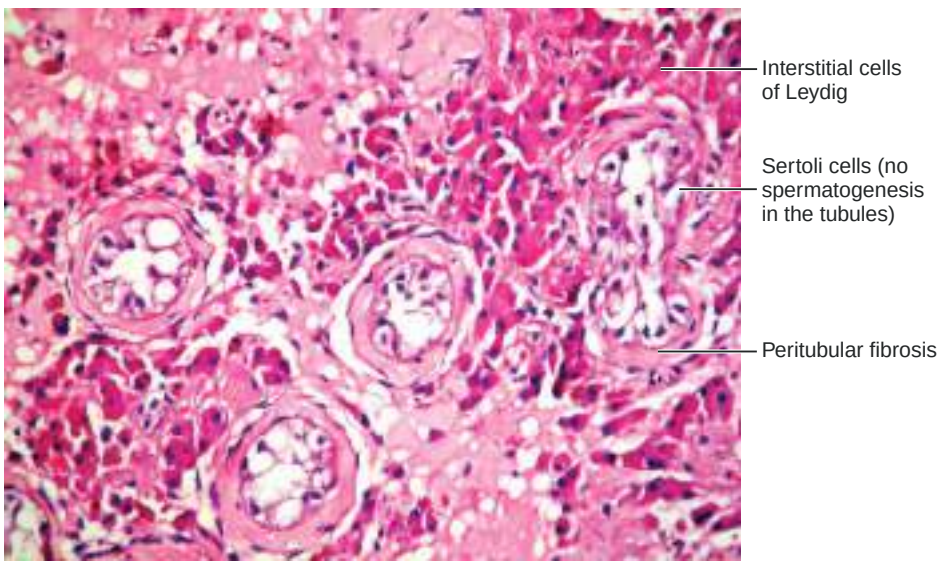


FIGURE 17.1. Atrophic testis showing marked loss of germ cells within the tubules, with peritubular and interstitial fibrosis with proliferation of interstitial cells of Leydig (H&E; 100×).

- Sertoli cells are present, but there is no spermatogenesis.
- Leydig cells are prominent.

Q. Classify testicular tumours. Describe their clinicopathological features.

Ans. Testicular tumours are classified based on their origin.

Classification of Testicular Tumours

1. **Germ cell tumours**
   (a) Seminomatous tumours
      (i) Classical semi as noma
      (ii) Spermatocytic seminoma
   (b) Nonseminomatous tumours
      (i) Yolk sac tumour
      (ii) Choriocarcinoma
      (iii) Embryonal carcinoma
      (iv) Germ cell tumours with multiple histological patterns, eg, embryonal carcinoma with teratoma and choriocarcinoma with others
   (c) Teratoma
2. **Sex cord-stromal tumours**
   (a) Sertoli cell tumours
   (b) Leydig cell tumours

Clinicopathological Features of Testicular Tumours

- All testicular tumours are derived from totipotent germ cells, which can show progressive and retrogressive differentiation; therefore, metastatic tumours may sometimes show a different histology as compared to the primary lesion, eg, an embryonal carcinoma presents as a teratoma in the metastatic lesion.
- Most testicular tumours are derived from intratubular germ cell neoplasia (ITGCN), which is also commonly seen in their vicinity. Exceptions are paediatric yolk sac tumour, teratomas and spermatocytic seminoma. ITGCN is found to be present as early as intrauterine life. It remains innocuous till puberty when it progresses to **seminomatous (SGCT)** or **nonseminomatous tumours (NSGCT)** subsequent to activating mutations, eg, reduplication of the short arm of chromosome 12 and kit activation. ITGCN is histologically characterized by presence of atypical primordial cells with large pleomorphic nuclei and clear cytoplasm.
- Germ cell tumours present as painless enlargement of testes. Their segregation into two categories (SGCTs and NSGCTs) is based on their different clinical behaviour.
- Biopsy of a testicular mass is associated with a risk of tumour spillage; therefore, any testicular mass is considered neoplastic unless proven otherwise and radical orchiectomy is performed based on this assumption.
- Lymphatic spread is common; retroperitoneal paraaortic lymph nodes are the first to be involved followed by mediastinal and supraclavicular lymph nodes.
- Haematogenous spread commonly involves lungs, liver, brain and bone.
- Germ cell tumours secrete polypeptide hormones and certain enzymes that can be detected in the blood, eg, AFP (α-fetoprotein), HCG (human chorionic gonadotrophins), PLAP (placental alkaline phosphatase), placental lactogen and LDH (lactate dehydrogenase).
- These hormones are useful in:
  - Diagnosis and staging of testicular germ cell tumours
  - Monitoring response to therapy
- Elevated levels of HCG and AFP are most often associated with NSGCTs (marked elevation is seen in yolk sac tumour and choriocarcinoma).
- Non-Hodgkin lymphoma is the most common testicular tumour after the fifth decade.
- Staging of testicular germ cell tumours:
  - Stage I: Tumours confined to testis, epididymis or spermatic cord

- Stage II: Spread confined to retroperitoneal lymph nodes below the diaphragm
- Stage III: Metastasis outside the retroperitoneal lymph nodes or above the diaphragm

Note: Most seminomas present in Stage I disease; lymph nodes are commonly involved; haematogenous spread is a late manifestation. Most NSGCTs present in Stage II or III disease; haematogenous spread is an early manifestation.

1. **Germ cell tumours**
   (a) **Seminomatous germ cell tumours (SGCTs)**
      (i) **Typical/classical seminoma (85%)**

***Clinical features*:**
- Most common type of germ cell tumour
- Peak age: third decade; never seen in infants
- Extremely radiosensitive

***Gross morphology*:**
- Classical seminomas are large tumours which may replace the entire testis but the testicular shape is maintained.
- Cut surface is homogeneous, grey-white and lobulated.
- Haemorrhage and necrosis are rare.
- Tunica albuginea is generally intact; however, occasional extension to epididymis, spermatic cord and scrotal sac may be seen.

Microscopy (Fig. 17.2):
- Sheets of monomorphic-looking seminoma cells are divided into poorly demarcated lobules by delicate fibrous septae.
- Seminoma cell is a large, round-to-polyhedral cell with a well-defined cell membrane; clear cytoplasm (due to glycogen or lipid contents), large central nucleus with one or two prominent nucleoli.
- Mitoses are infrequent.
- Septae are infiltrated by T lymphocytes; at times granulomas may form.

***Immunochemistry*:**
- Tumour cells stain positive for PLAP, kit and OCT 4.
- HCG is positive in 15% cases where syncytial giant cells resembling syncytiotrophoblasts of placenta are present.

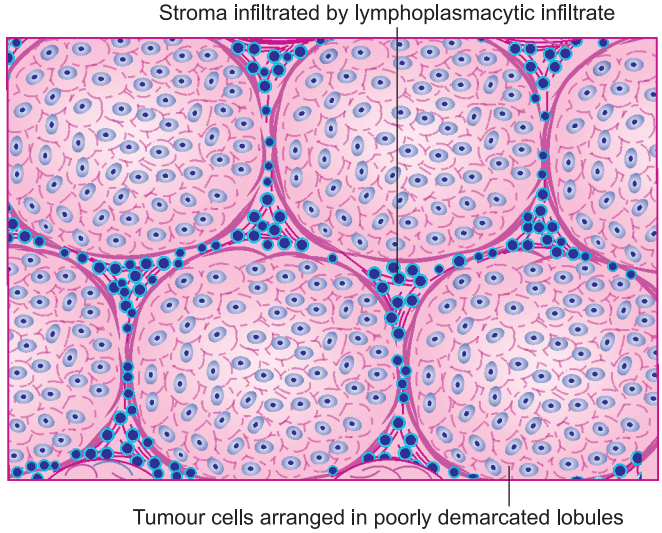


FIGURE 17.2. Section from seminoma testis showing sheets of large, round-to-polyhedral cells with well-defined cell membrane; clear cytoplasm, large central nucleus and one or two prominent nucleoli. The sheets are divided into poorly demarcated lobules by delicate fibrous septae which are infiltrated by T lymphocytes.

(ii) **Anaplastic seminoma (5–10%):** Shows greater cellularity, more nuclear irregularity, a larger number of tumour giant cells and three or more mitoses per high power field (anaplastic seminoma is not treated differently from a typical seminoma because it does not have a worst stage-by-stage prognosis as compared to the same)

(iii) **Spermatocytic seminoma (4–6%):** Classified separately due to differences in clinicopathological profile when compared with a classical seminoma. It has the following features:

***Clinical presentation*:**
- Presents as a large testicular swelling
- Peak age: More than 65 years
- Slow-growing tumour that rarely metastasizes; has excellent prognosis

***Gross morphology*:** Larger than typical seminoma; cut surface is pale grey, soft and friable

***Microscopy*:**
- Composed of three distinct cell populations:
  - Medium-sized cells (round nucleus and eosinophilic cytoplasm)
  - Smaller cells (resemble secondary spermatocyte; have scanty eosinophilic cytoplasm)
  - Scattered giant cells (uninucleate or multinucleate)
- Lacks lymphocytes, granulomas and syncytiotrophoblasts

(b) **Nonseminomatous germ cell tumours (NSGCTs)**

(i) **Yolk sac tumour**
- Also called **endodermal sinus tumour, orchioblastoma** and **infantile embryonal carcinoma**
- Most common testicular tumour of infants and children up to three years of age
- The pure form is uncommon in adults, in who it frequently occurs in combination with embryonal carcinoma.
- AFP level is elevated in all cases of yolk sac tumour.

***Gross morphology*:** Unencapsulated; cut surface is yellow-white, mucoid with area of necrosis, haemorrhage and microcyst formation

***Microscopy*:**
- Tumour cells are flattened to cuboidal with clear to vacuolated cytoplasm, arranged in a variety of patterns varying from loose, lace like or reticular to tubular, tubulopapillary and solid.
- Cells may form distinct **perivascular structures,** ie, a central blood vessel or mesodermal core surrounded by germ cells arranged in visceral and parietal layers like glomeruli **(resemble yolk sac or endodermal sinus of rat placenta called Schiller–Duval bodies).**
- Intracellular and extracellular PAS-positive hyaline globules may be present.
- Tumour cells may also contain AFP and α_1-antitrypsin.

(ii) **Choriocarcinoma**
- Highly malignant form of testicular cancer
- May arise in placental tissue, ovaries, mediastinum and abdomen (from sequestered totipotential cells)
- Pure form of choriocarcinoma is rare; mostly mixed tumours
- The serum and urinary levels of HCG are greatly elevated in all cases.

***Gross morphology*:**
- Generally, does not cause testicular enlargement, detected only as a small palpable nodule.
- Areas of haemorrhage and necrosis are extremely common.
- Tumour may undergo extensive ischaemic necrosis to be eventually replaced by a fibrous scar leaving behind extensive metastasis.

***Microscopy*:** Two types of cells are seen without formation of placental type villi, namely:
- ***Syncytiotrophoblasts*:** Large, multinucleated cells with irregular or lobular hyperchromatic nuclei and abundant eosinophilic cytoplasm; HCG is localized to their cytoplasm.

- ***Cytotrophoblasts*:** Regular, polygonal cells with distinct cell borders, clear cytoplasm; single uniform nucleus; grow in cords and masses.

(iii) **Embryonal carcinoma**
- Common in third decade
- Tumour is composed of markedly pleomorphic cells, arranged in tubules, acini or sheets.
- Tumour cells have hyperchromatic nuclei with prominent nucleoli.
- Necrosis is prominent.
- Tumour secretes AFP and HCG.

(c) **Teratoma**
- Tumour composed of differentiated tissue derived from more than one germ cell layer arranged in a haphazard but organoid pattern in a fibrous or myxoid stroma.
- More common in infants and children (constitutes 40% of infantile testicular tumours). Teratoma in a prepubertal child is considered benign, whereas that in a post-pubertal male is regarded as malignant.
- A large number of these are mixed tumours (most commonly occur in combination with embryonal carcinoma).
- Elevated HCG or AFP is found in 50% cases.

***Gross morphology*:** Large tumour, may replace the whole testis.

***Cut surface*:** Variegated appearance—grey-white with solid and cystic areas; may show foci of cartilage and bone formation.

***Microscopy*:**

Based on histology, teratomas are classified into three types:
- Mature (differentiated) teratoma
  - Composed of a variety of well-differentiated (resembling adult tissue) structures like cartilage, bone, smooth muscle, intestinal and respiratory epithelium, mucous glands, thyroid, bronchial, bronchiolar and transitional epithelium, neural tissue and fat.
  - The cystic variant with primarily ectodermal differentiation is labelled 'dermoid cyst' and is more common in ovaries than testes.
- Immature teratoma: Characterized by the presence of elements resembling foetal or embryonal tissue.
- Teratomas with malignant transformation: Clear evidence of a non–germ cell malignancy arising in the derivatives of one or more germ cell layers; usually squamous cell carcinoma, adenocarcinoma or a sarcoma.

2. **Sex cord-stromal tumours**

(a) **Sertoli cell tumours**

(i) Yellowish, homogenous cut surface

(ii) Histologically, show small cells arranged in trabeculae or cords resembling immature seminiferous tubules

(iii) Associated with hormonal effects

(b) **Leydig cells**

(i) Derived from and resemble normal testicular interstitial cells

(ii) Well-defined nodules < 5 cm in diameter

(iii) Characteristic golden brown colour due to intracytoplasmic inclusions called **Reinke's crystalloids** and **lipofuscin.**

Q. Differentiate between seminomatous and nonseminomatous germ cell tumours.

Ans. Differences between the seminomatous and nonseminomatous germ cell tumours are shown in Table 17.1.

TABLE 17.1. Differences between the seminomatous and nonseminomatous germ cell tumours

Features	SGCTs	NSGCTs
Components	Only one histological type; secrete HCG in 15% cases	Umbrella designation that includes one histological type as well as more than one histological type or mixed tumours; secrete HCG, AFP, LDH, PLAP, HPL, etc.
Spread	• Remain localized to testes for a long time • Mainly metastasize to lymph nodes; haematogenous spread late	• Metastasize early • Haematogenous spread early and more frequent
Stage	Majority present in Stage I	Majority present in Stages II and III
Gross	• Areas of necrosis and haemorrhage are rare • Less tendency to infiltrate tunica, epididymis, spermatic cord and scrotal sac • Shape of the testis is maintained	• Necrosis and haemorrhage are common • Greater tendency to infiltrate tunica, epididymis, spermatic cord and scrotal sac • Shape of the testis may be distorted
Response to radiation	Radiosensitive	Radio resistant
Behaviour	Less aggressive	More aggressive
Prognosis	Good	Bad

PENIS

Normal Structure

- The penis consists of three cylindrical erectile vascular tissue bodies (two corpora cavernosa of the penis, placed dorsally and one corpus cavernosum of the urethra, placed ventrally), all covered by skin.
- The longest part of the penis is labelled the shaft, at the end of which is the head, or glans penis. The *frenulum* or *frenum* is a connecting membrane on the underside of the penis
- The glans has a covering, called the foreskin or prepuce. The prepuce is a retractile fold of skin containing connective tissue, smooth muscle and sebaceous glands.

Q. Write briefly on the inflammatory conditions affecting penis.

Ans. Salient features of inflammatory conditions affecting penis:

- The foreskin of the penis (prepuce) and the glans penis (the conical end of the penis) are the areas usually affected by inflammation. Inflammation of the glans and foreskin are labelled **balanitis** and **posthitis**, respectively. **Balanoposthitis** is inflammation of both the glans penis and the foreskin.
- Causes of penile inflammation include **infectious** and **noninfectious conditions.**
- **Common infectious causes** are yeast infections (*Candida albicans*), sexually transmitted diseases (gonorrhoea, herpes and syphilis) and scabies.
- **Noninfectious causes** include *allergic reactions* (to latex condom or to contraceptive gels), *papulosquamous disorders* (lichen planus, psoriasis), *seborrheic dermatitis* and *balanitis xerotica obliterans* (chronic inflammation of the glans which results in formation of white plaques on the foreskin and glans, which on histopathology show changes similar to lichen sclerosus et atrophicus and may lead to constriction of the urinary passage).
- Penile inflammation may manifest with pain, swelling, irritation, redness, erosions/ulceration and enlarged groin lymph nodes.

- Balanoposthitis patients are predisposed to **phimosis** (narrowing of the preputial orifice resulting in nonretraction of the preputial skin over the glans) and penile cancer.
- Diagnosis and typing of inflammatory conditions of penis entails the following steps:
  1. Physical examination
  2. Blood sugar measurement (for diabetes)
  3. KOH mount and culture for yeast infections
  4. Specific tests for STDs

Q. Write briefly on the neoplasms involving penis.

Ans. Following are the commonly encountered benign and malignant lesions involving penis:

1. **Condyloma acuminatum**
   - Also known as 'anogenital wart', it is associated with HPV 6 and 11 and may present as a solitary or multiple lesions. Common sites are the coronal sulcus of the penis and the perianal area.
   - Anogenital wart has a large cauliflower-like exophytic variant labelled **'Buschke–Lowenstein tumour' (Verrucous carcinoma).**
   - Microscopy shows papillary projections composed of a connective tissue core lined by squamous epithelium. The epithelium shows hyper-/parakeratosis with acanthosis of the stratum malphgium. Koilocytosis is the histopathologic hallmark.
2. **Premalignant conditions**
   - **PeIN** can occur on the glans or foreskin of the penis (**erythroplasia of Queyrat)** or on the shaft (**Bowen disease)**. Erythroplasia of Queyrat and Bowen disease have similar clinical behaviour and are both associated with HPV. The former is common in uncircumcised men and presents as reddish and velvety pigmentation on the glans. **Bowen disease** is characterized by well-marginated, reddish plaques over the shaft of penis which may ulcerate and crust.
   - **Bowenoid papulosis** is histopathologically identical to the above two entities (Bowen disease and erythroplasia of Queyrat) and all show severe dysplastic changes on biopsy. Clinically, 'Bowenoid papulosis' is associated with HPV 16 and presents as multiple reddish verrucous papules**.**
3. **Carcinoma penis**
   ***Salient features*:**
   - Almost all penile cancers are squamous in origin.
   - The overall incidence is less than 1% of all cancers of the male.
   - It has an established causal association with high-risk HPV types (16 and 18).
   - It is more common in blacks and rare in Jews and Muslims who customarily undergo circumcision (as circumcision prevents accumulation of smegma which is thought to be carcinogenic).
   - Carcinoma penis usually affects men over 50 years.

   ***Gross morphology*:**
   - The tumour may be exophytic (papillary or cauliflower type) or ulcerative.
   - Usual locations are the fraenum, prepuce, glans and the coronal sulcus, in that order.

   ***Microscopy*:**
   In most cases, sections show a well-to-moderately differentiated squamous cell carcinoma, which commonly metastasize to the regional lymph nodes as well as viscera.

PROSTATE

Normal Structure

- The prostate weighs about 20 gm in a normal adult. It surrounds the beginning of the male urethra and has 3 lobes—a median and two lateral.
- Histologically it is constituted by 30–50 branched acini (tubule-alveolar structures) lying in a fibromuscular stroma. The acini are lined by two layers, a basal cuboidal cell layer and an inner layer of mucous-secreting columnar cells.

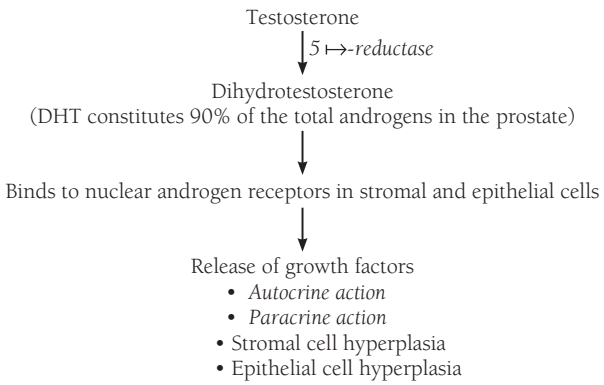
- The prostate is divided into two parts depending on the hormone responsiveness, the inner periurethral part which is sensitive to oestrogen and androgens and an outer subcapsular part that is sensitive to androgen.

Q. Write briefly on the aetiopathogenesis, clinical features and morphology of nodular hyperplasia of prostate (NHP).

Ans. NHP (benign prostatic hyperplasia) is defined as hyperplasia of prostatic stromal and epithelial cells, resulting in the formation of discrete nodules in the transitional and inner periurethral zones (prostate is divided into several zones namely, peripheral, central, transitional and periurethral). The nodules compress the prostatic urethra to produce the clinical symptoms of NHP.

- NHP was earlier called 'benign hypertrophy', which is a misnomer because the fundamental lesion is a hyperplasia and not hypertrophy.
- **Age group** affected is more than 50 years; incidence increases with increasing age.

Pathogenesis (Flowchart 17.1)



FLOWCHART 17.1. Pathogenesis of NHP.

- **Testosterone** is converted into **DHT by 5α-reductase type II enzyme specifically located in the stromal cells.**
- **DHT is 10 times more potent than testosterone**, as it dissociates slowly from androgen receptors.
- DHT thus produced, acts on **nuclear receptors to produce growth factors** that are mitogenic to **epithelial and stromal cells.**
- Testosterone acts similarly, but is **very weak.**
- **Oestrogen** increases the expression of androgen receptors, thus providing DHT more sites for action. Oestrogen levels increase with age, making its role significant.

Clinical Features

- **Clinical symptoms are seen in 10% of affected patients.**
- **Early changes:** Compression of urethra leading to increased frequency, nocturia (urgency), problem in starting and stopping the stream of urine, overflow dribbling and painful micturition.
- **Late complaints:** Retention of urine in the bladder causing urinary tract infection (UTI), cystitis, hypertrophy or trabeculation in urinary bladder and *hydronephrosis* may occur.

Gross Morphology

- Affected gland is enlarged; may weigh 300 gm or more.
- Cut surface shows multiple well-defined nodules that bulge from the surface.
- Two types of proliferation, **glandular** and **stromal (fibromuscular)**, are seen.
- In nodules with primarily *glandular* proliferation, the cut surface is yellow-pink; consistency is soft with milky-white fluid oozing out of it.
- In nodules with primarily *stromal (fibromuscular)* proliferation, surface is pale-grey, tough without any fluid oozing out.
- True capsule is absent but plane of cleavage is present.

Microscopy (Fig. 17.3 A and B)

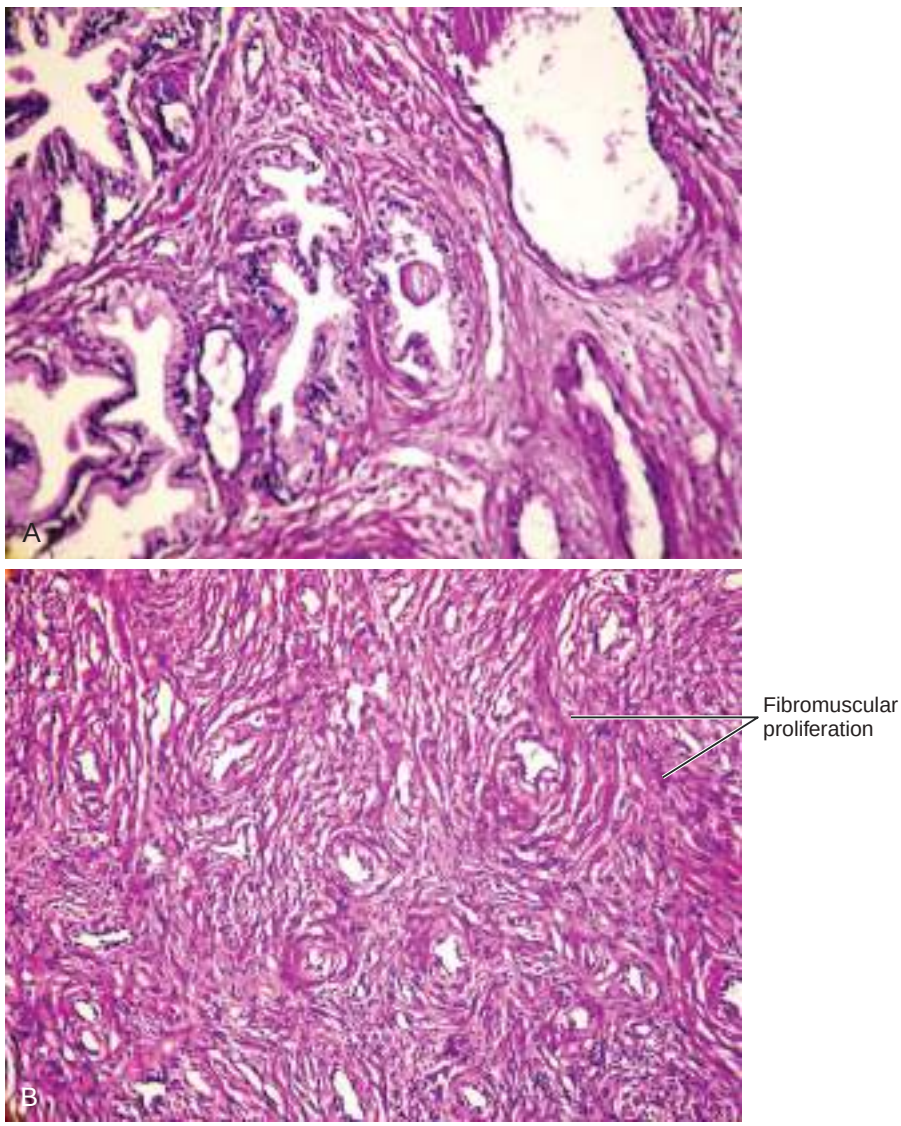


FIGURE 17.3. (A) Section from NHP showing proliferation and cystic dilatation of glands lined by two layers—inner layer of columnar cells and outer layer of cuboidal or flattened epithelium. Multilayering and crowding of epithelium with formation of papillary infoldings is also seen (H&E; 100×). (B) Section from NHP showing stromal hyperplasia (H&E; 100×).

Glands

- There is proliferation and cystic dilatation of glands, which are lined by two layers: inner layer of columnar cells and outer layer of cuboidal or flattened epithelium. Basement membrane is intact. Multilayering and crowding of epithelium leads to the formation of papillary infoldings.

Stroma

- Glandular proliferation is accompanied by the fibrous and muscular proliferation.
- Squamous metaplasia and small foci of infarction may be present.

Q. Describe the aetiopathogenesis, clinical features and morphology of the carcinoma prostrate.

Ans. Carcinoma prostate is the most common form of visceral cancer (followed by the lung cancer), and second leading cause of death in males.

Clinical Features

- Usually affects men over 50 years
- Seventy to eighty percent arises in the **peripheral zone**; due to its peripheral location, it is less likely to cause urethral obstruction in the early stages.
- Most cases are clinically silent; a few are discovered accidentally in prostatic tissue removed for NHP.
- Extensive prostatic disease can produce 'prostatism' (local discomfort and lower urinary tract obstruction).
- May come to attention due to bone metastases (may be lytic, more commonly blastic).

Factors Implicated in the Pathogenesis

- **Dietary factors:** Increased consumption of fats and reduced consumption of lycopenes, selenium, soya products and vitamin D increase the risk of prostatic cancer.
- **Family history:** Men with a family history of prostatic cancer have a twofold increase in incidence and an earlier age of onset.
- **Genetic factors:**
  - Prostatic carcinoma is initially androgen dependent and relies on the androgen receptor (AR) to mediate the effects of androgens (therapy includes antiandrogens and LHRH analogues). However, all cancers eventually become androgen independent, often referred to as hormone refractory prostate cancer. This transformation is not yet fully understood (AR amplification, overexpression or mutation and alterations in the AR signalling pathway may play a role).
  - Analyses have revealed that **hypermethylation of GSTP1 (glutathione S-transferase P) gene**, encoding the carcinogen detoxification enzyme glutathione S-transferase pi, may serve as an initiating genome lesion for prostatic carcinogenesis. Somatic mutation leading to juxtaposition of coding sequence of **ETS family transcription factor gene** next to **androgen-regulated TMPRSS2 promoter** induces overexpression of ETS transcription factors which upregulates matrix metalloproteinases to enhance invasiveness of prostatic cancer cells.
  - Germline mutations of BRCA2 are associated with a twentyfold increase in the risk.
  - Mutations and deletions which activate PI3K/AKT signalling pathway are commonly involved. There is amplification of 8q24 locus containing the MYC oncogene as well as deletions affecting the PTEN, P53 and RB tumour suppressor genes.

Gross Morphology

- Prostate is enlarged, normal sized or smaller than normal, hard and fixed.
- Cut section is homogeneous, fibrous and may show yellowish irregular areas.

- Local invasion into seminal vesicles, adjacent soft tissue and wall of the urinary bladder may be seen.
- Invasion of rectum is less common (Denonvilliers, fascia separating the lower urinary tract structures from the rectum, prevents growth into the rectum).

Microscopy

Four histological types:
1. Adenocarcinoma
2. Transitional cell carcinoma
3. Squamous cell carcinoma
4. Undifferentiated carcinoma

Adenocarcinoma Prostate

- It is the most common histological type (96% cases).
- The tumour is composed of closely packed acini arranged in a back-to-back manner with little or no stroma between them.
- Glands may be well differentiated to almost undifferentiated and are lined by a single layer of epithelium (basal layer seen in normal or hyperplastic glands is absent). Tumour cells may be clear, hyperchromatic or eosinophilic (granular).
- Foci of intraepithelial neoplasia (PIN) may be seen in close association with carcinoma.
- Invasion of intraprostatic perineural spaces is a common occurrence.

Grading of Carcinoma Prostate

Gleason grading is the most widely used grading system for adenocarcinoma prostate. It is based on the glandular architectural patterns and the relationship of the tumour cells with the stroma.

Diagnosis and Staging of Carcinoma Prostate

- **Digital rectal examination:** Most of the prostatic tumours are located in posterior lobe, so are easily palpable on per rectal examination.
- **Transrectal ultrasonography with guided biopsy** for early detection of tumour.
- **Computed tomography and magnetic resonance imaging scan** to evaluate the lymph node status.
- **Pelvic lymphadenectomy** to look for microscopic metastasis as metastasis to regional pelvic lymph nodes can occur.
- **Skeletal survey or radionuclide scanning** for detection of osteoblastic metastasis.
- **Tumour marker assays:**
  - ***Prostatic acid phosphatase (PAP)*:**
    - Secreted by normal as well as cancerous prostatic epithelial cells.
    - Serum level is highly raised in prostatic cancer extending beyond the capsule or in metastases.
    - Normal values: 1–3 KA° units, more than 5 KA° unit is diagnostic of the cancer.
  - ***Prostate-specific antigen (PSA)*:**
    - Produced by the prostatic epithelium and secreted in small quantities in the serum, PSA cleaves and liquefies seminal coagulum by its enzymatic activity (androgen-regulated serine protease).
    - Any condition that disrupts the normal architecture of prostate, whether adenocarcinoma, NHP or prostatitis, can elevate serum levels of PSA.
    - A serum PSA of more than 4 ng/mL is most useful in diagnosing prostatic cancer; particularly, in combination with rectal examination and transrectal ultrasonography.
    - PSA levels are generally higher in cancer as compared to nodular hyperplasia, but their values may overlap, so criteria other than simply serum levels to be looked for; namely, **free PSA levels** (levels lower than 10% is indicative of prostatic cancer

whereas levels greater than 25% indicates a low risk of cancer). Other indicators include
  - **Ratio of free and bound PSA**
  - **PSA density** (ratio between the serum PSA levels and volume of prostate gland)
  - **PSA velocity** (ratio of change in PSA value with time)
- Uses of PSA
  - Diagnosis of prostatic diseases
  - To assess the response to chemotherapy in cancer
  - To check whether radical prostatectomy is complete or not
  - To confirm the origin of metastatic deposits as prostate
- ***EZH2 (enhancer of zeste 2)*:** Loss of E-cadherin (adhesion protein) from prostatic cancer cells is associated with high levels of EZH2 and may contribute to prostatic cancer progression
- ***AMACR*** (α methylacyl-CoA racemase): AMACR is an enzyme involved in beta-oxidation of branched amino acids and is upregulated in prostatic cancer compared to normal prostate
- ***PCA3*:** A gene on chromosome 9q that possibly encodes a regulatory RNA; it is over-expressed in >90% cases of prostatic carcinoma.

18

Female Genital System

NORMAL ANATOMY

Uterus has three anatomical and functional regions:

1. Cervix
2. Lower uterine segment
3. Corpus

Cervix

Cervix is further divided into:

- **Ectocervix (**vaginal portion**):** It is the part of cervix that is visible from the vaginal canal. Ectocervix is lined by nonkeratinizing stratified squamous epithelium continuous with the vagina. The squamous epithelium converges centrally at a small opening called **external os,** which is closed in nulliparous women.
- **Endocervix:** It is lined by columnar mucous secreting epithelium, which meets the squamous epithelial covering at the **squamocolumnar junction**. The endocervical stroma contains endocervical glands.

Progressive differentiation of the subcolumnar reserve cells determines the position of the squamocolumnar junction. The portion of the columnar epithelium ultimately replaced by the squamous epithelium is termed the **transformation zone,** which is important clinically because this is where the precancerous and cancerous lesions develop.

Lower Uterine Segment and Corpus

Uterus has two main components—the **endometrium** and the **myometrium.** Endometrium is composed of glands embedded in a stroma and the myometrium is composed of interwoven smooth muscle bundles. The **endometrial cavity (internal cavity of uterus)** is slit like and best opened by inserting a probe and cutting along with it. The **endometrium** is 1–5 mm wide and the **myometrium** is 1–2 cm wide. From each side of uterus, broad ligament arises, which is inserted into the lateral wall of the pelvis. Fallopian tube travels in the free border of broad ligament.

Ovaries

- Surface is lined by germinal epithelium and the average size is 4 cm × 1.5 cm × 1.5 cm.
- It is divided into **cortex** and **medulla.** Cortex consists of closely packed stromal cells with a thin covering of collagen.
- Outer cortex shows follicles in varying stages of maturation. In each menstrual cycle, one follicle develops into **Graffian follicle,** which is transformed into a **corpus luteum** after ovulation. Senescent corpus luteum is called **corpora albicans.**
- Medulla consists of loosely arranged mesenchymal tissue and contains remnants of **Wolffian duct** as well as round to polygonal epithelioid cells around vessels and nerves called **Hilus cells.**

Fallopian Tube

- Average size is 10 cm × 1 mm; it is divided into four anatomical regions, namely, isthmus, ampulla, fimbriae and abdominal opening.
- Mucosa is lined by three cell types—ciliated columnar, nonciliated columnar and intercalated cells (inactive secretory cells).
- Anterior to the tubes there is insertion of **round ligament.** Most lateral portion of broad ligament is called **infundibulopelvic** or **suspensory ligament** and transmits ovarian vessels and nerves.

Nulliparous Uterus

- Weighs 30–40 g, with an average size of 7.5 cm × 5 cm × 2.5 cm.
- It has an inverted flattened pear appearance with the presence of a constriction called isthmus.
- Anterior peritoneal reflection is at isthmus; posteriorly peritoneum covers the entire uterus and passes down to cover the upper portion of the vagina.
- Vaginal portion is covered by moist, smooth vaginal epithelium.
- Cervical canal is narrow and fusiform.

Multiparous Uterus

Weighs 60–70 g, with an average size of 10 cm × 5 cm × 4 cm.

Postmenopausal Uterus

- Atrophic/more fibrous
- Cervix less prominent
- Uterus is anteflexed (sharply bent forward upon vagina)

Q. Write briefly on cervical intraepithelial neoplasia (CIN).

Ans. CIN is a precancerous lesion frequently associated with HPV infection. HPV is a sexually transmitted DNA virus which can lead to CIN as well as invasive squamous cell carcinoma. The spectrum of changes associated with progression of CIN to invasive carcinoma is illustrated in Figure 18.1. According to Bethesda system, precancerous lesions of cervix are divided into two groups:

- **Low-grade squamous intraepithelial lesion or LSIL (CIN I):** Associated with HPV types 6, 11, 42 and 44 (also known as HPV types with low oncogenic potential).
- **High-grade squamous intraepithelial lesion or HSIL (CIN II and III):** Associated with HPV types 16, 18, 31, 33 and 45 (also known as HPV types with high oncogenic potential).

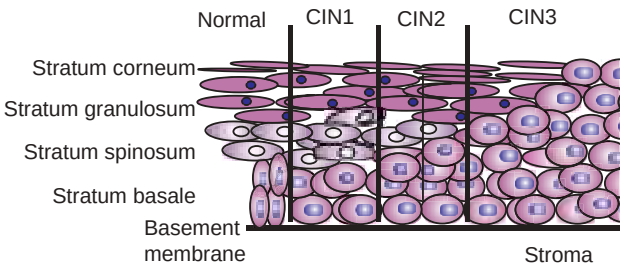


FIGURE 18.1. Histopathological spectrum of CIN.

CIN I

- Dysplasia is present in lower one-third of stratified squamous epithelium.
- May be raised (as in condyloma acuminatum) or macular (flat condyloma).
- Abundant HPV nucleic acid of low-risk HPV type is present.
- Koilocytic atypia or viral cytopathic effect is seen (koilocytosis is seen as nuclear abnormalities with perinuclear halo).

CIN II

- Dysplasia is limited to basal two-thirds of stratified squamous epithelium.
- Increased number of atypical cells in lower layers (increased N/C ratio, anisokaryosis, loss of polarity, mitotic figures and hyperchromasia)
- Upper layer cells appear differentiated
- Associated with high-risk HPV types

CIN III/Carcinoma In Situ

Dysplasia spreads to the entire thickness of the epithelium.

Note: A low-grade lesion does not always progress to a high-grade lesion. Most low-grade lesions regress spontaneously; whereas, most high-grade lesions progress.

Q. Write briefly on predisposing factors, aetiology, morphology and diagnosis of carcinoma cervix.

Ans. Carcinoma cervix is a major cause of morbidity and mortality.

Predisposing Factors for Carcinoma Cervix

- ***HPV infection:*** Nearly all cervical carcinoma is HPV related (Types 16, 18, 31, 45, etc.).
- Early age at first intercourse
- Multiple sexual partners or a male partner with multiple sexual partners
- Oral contraceptives
- Cigarette smoking
- High parity
- Family history
- Associated genital infections
- Lack of circumcision in male sexual partner

Pathogenesis

Sequence of events that follow HPV infection are given in Flowchart 18.1.

Clinical Features

- Usually affect women between fourth and sixth decades
- Present with unexpected vaginal bleeding, leucorrhoea, painful coitus (dyspareunia) and dysuria (due to bladder infiltration).

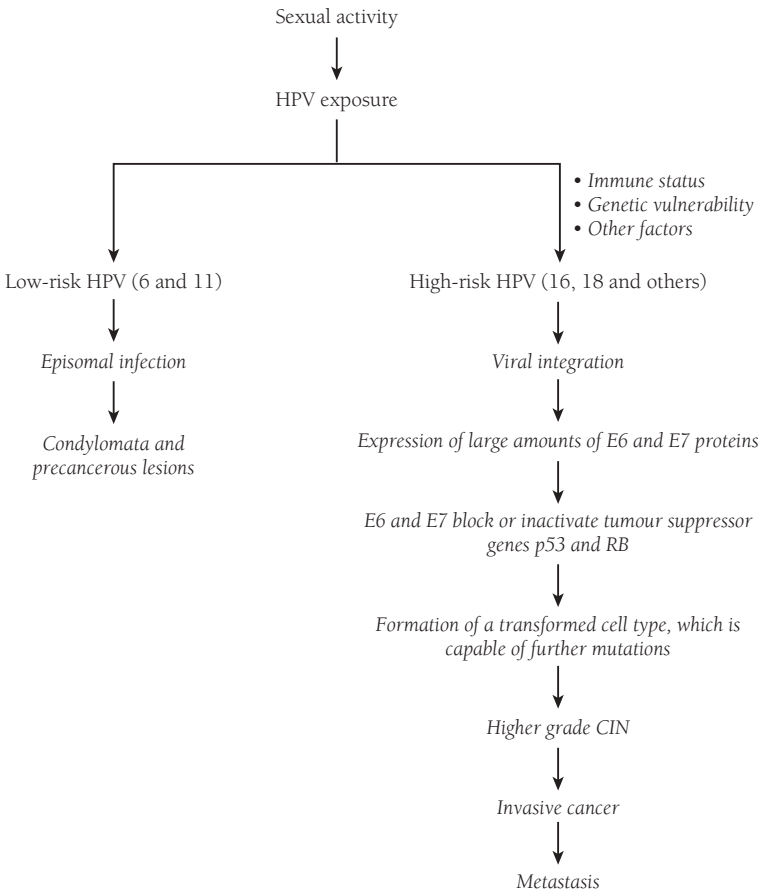
Gross Morphology

Arises from the transformation zone and has three main gross types:

- Fungating or exophytic (most common)
- Ulcerative or ulceroinfiltrative
- Infiltrative

Microscopy

- Most (90%) are squamous cell carcinomas (SCCs)



FLOWCHART 18.1. Sequence of events that follow HPV infection.

- Subtypes include
  (a) Large cell keratinizing—Nests of keratinized cells which form concentric whorls known as keratin pearls (Fig. 18.2).
  (b) Large cell nonkeratinizing—Nests of large malignant squamous cells which show individual cell keratinization but no keratin pearls.
  (c) Small cell carcinoma—This type is the least common but has the most aggressive course; it is composed of small nonkeratinized malignant cells.

Other Morphologic Types of Carcinoma Cervix

- Adenocarcinoma
- Adenosquamous carcinoma
- Small cell neuroendocrine carcinoma
- Undifferentiated carcinoma

Spread and Staging

- Stage 0: Carcinoma in situ (CIN III, HSIL)
- Stage I: Carcinoma confined to the cervix:
  - 1a: Preclinical carcinoma diagnosed only by microscopy
  - $1a_1$: Minimally invasive carcinoma (invasion of stroma not deeper than 3 mm and not wider than 7 mm)
  - $1a_2$: Microscopic invasion of stroma more than 3 mm and not deeper than 5 mm; horizontal invasion not more than 7 mm
  - 1b: Histologically invasive carcinoma of cervix greater than stage $1a_2$

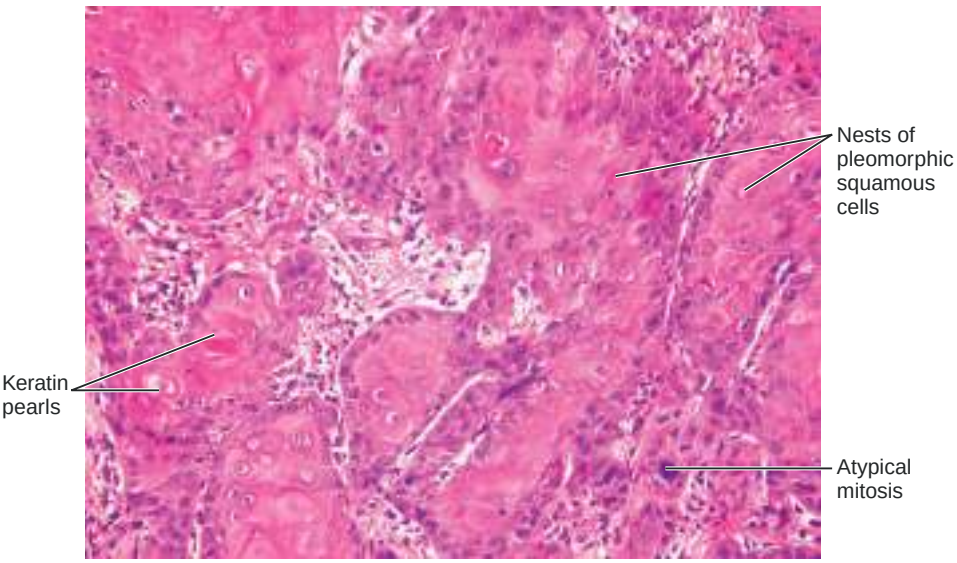


FIGURE 18.2. H&E-stained section from a large cell keratinizing squamous cell carcinoma cervix showing nests of pleomorphic squamous cells with keratin pearls (H&E; 200X).

- Stage II: Carcinoma extends beyond the cervix but pelvic wall is not involved. Carcinoma involves vagina but without the involvement of its lower third.
- Stage III: Pelvic wall and lower one-third of vagina are also involved by carcinoma. On digital rectal examination, there is no cancer-free space between the tumour and the pelvic wall.
- Stage IV: Extension of carcinoma beyond pelvic wall. May involve mucosa of bladder or rectum, or show systemic metastasis.

Diagnosis and Prevention

- Pap smear examination is the most important tool for screening of carcinoma cervix. It entails cytological examination of exfoliated cervical cells after staining them with Papanicolaou method. The transformation zone is scraped with an Ayer's spatula or a cytological brush to obtain the material.
- Also, HPV DNA testing can be done to assess the HPV status of the patient.
- In case of an abnormal Pap smear, colposcopic examination of the cervix and vagina is performed to determine the extent of the lesion. The lesion is then biopsied. Application of acetic acid may also highlight abnormal areas.
- LSIL is generally followed up by repeated Pap smear examination and HSIL is excised by conization and follow-up pap smears.
- Prophylactic HPV vaccine for HPV subtypes 6, 11, 16 and 18 is now available.

Q. Define adenomyosis.

Ans. Growth of endometrial tissue into the myometrium is called adenomyosis. Clinical features of adenomyosis include irregular, heavy menses and pelvic pain. Microscopy shows the presence of nests of endometrial glands and/or stroma well down in the myometrium between the muscle bundles. The endometrial tissue must be separated from the basalis by at least 2–3 mm.

Q. Define endometriosis. Enumerate the theories that are proposed to explain its origin.

Ans. Endometriosis is the presence of endometrial glands and/or stroma in abnormal locations outside the uterus. It is seen in the reproductive age group and mostly manifests in the third and fourth decades.

Sites Involved (in Decreasing Order of Frequency)

- Ovaries
- Uterine ligaments
- Recto vaginal septum
- Pelvic peritoneum
- Laparotomy scar
- Rarely, in umbilicus, vagina, vulva, appendix

Pathogenesis

Several theories have been proposed to explain endometriosis:

- ***Regurgitation/transplantation theory*:** According to this theory, endometriosis occurs due to regurgitation of menstrual blood through fallopian tubes which transports endometrial tissue from uterus to peritoneal cavities and other locations. This is the most widely accepted theory as it explains the genesis of most cases of endometriosis.
- ***Metaplastic theory*:** As per this theory, coelomic epithelium gives rise to endometrial tissue by metaplasia.
- ***Benign metastasis (vascular or lymphatic dissemination) theory*:** It explains the presence of endometrial tissue at extra pelvic sites like lung or lymph nodes.
- ***The extrauterine stem or progenitor theory*:** This relatively new theory proposes that the extrauterine endometrial tissue arises from stem cells derived from bone marrow.

Molecular analysis has shown that endometriotic implants release proinflammatory factors (IL1β, TNFα, IL6, IL8, PGE2, NGF, VEGF, MCP1, MMPs and TIMPs), which increase oestrogen levels (PGE2 stimulates local synthesis of oestrogen), promote invasion and increase survival of extrauterine endometriotic tissue by reducing its immune clearance. Also, high levels of an enzyme 'aromatase' have been demonstrated in these implants which also contribute to increase oestrogen production.

Clinical Features

- Dysmenorrhoea
- Dyspareunia
- Infertility
- Pelvic pain due to intrapelvic bleeding and periuterine adhesions
- Pain on defecation or urination (due to involvement of bowel or bladder)

Gross Morphology

- Red-yellow-brown, often bilateral, nodules present on or just beneath the serosal surface of the sites involved.
- Extensive haemorrhage may cause fibrotic adhesions of different layers.
- Large cystic space filled with brown bloody debris, may distort ovaries (***chocolate cysts***).

Prerequisites for a Histological Diagnosis of Endometriosis

Two of the three following features must be present for a diagnosis of endometriosis:

- Endometrial glands
- Endometrial stroma
- Haemosiderin-laden macrophages

Atypical Endometriosis

Endometriosis is said to be atypical if the epithelium lining the endometriotic cyst shows atypia without architectural distortion or if there is architectural distortion (glandular crowding) with atypia.

Atypical endometriosis and endometrioid and clear cell types of endometrial carcinoma share PTEN and AT-rich interactive domain-containing protein (ARIDA1) mutations suggesting that endometriosis may be a precursor to carcinoma.

Q. Classify endometrial hyperplasia. Write briefly on its different types.

Ans. Proliferation of glandular and stromal tissue, associated with prolonged, profuse and irregular uterine bleeding in menopausal or postmenopausal women is known as endometrial hyperplasia.

Classification

International Society for Gynaecological Pathology classifies endometrial hyperplasia into:

1. Simple hyperplasia without atypia
2. Simple hyperplasia with atypia
3. Complex hyperplasia without atypia
4. Complex hyperplasia with atypia

WHO has recently recommended that endometrial hyperplasia should be classified into two major categories - **"non-atypical hyperplasia"** and **"atypical hyperplasia (endometrial intraepithelial neoplasia)"**.

Gross Morphology

Diffuse thickening of endometrium with a velvety appearance or focal overgrowth, which may be mistaken for a polyp.

Microscopy

Increase in endometrial glands relative to the stroma.

1. International Society for Gynaecological Pathology Classification

Simple Hyperplasia Without Atypia

- Cystically dilated glands with occasional outpouching (**Swiss cheeses appearance**)
- Mild increase in gland to stroma ratio and focal crowding of glands
- Epithelial morphology resembles proliferative endometrium
- Thought to be due to persistent oestrogenic influence and less than 1% progress to endometrial carcinoma

Simple Hyperplasia With Atypia

- Architecture is like simple hyperplasia but there is presence of cellular atypia, eg, loss of polarity, open chromatin and prominent nucleoli.
- Approximately, 8% progress to endometrial carcinoma.

Complex Hyperplasia Without Atypia

- Complex crowded glands with branching and minimal intervening stroma
- Epithelial stratification (2–4 layers)
- Mitotic activity (5–10 mitotic figures/10 HPF)
- No cytological atypia
- Approximately, 3% progress to endometrial carcinoma

Complex Hyperplasia With Atypia

- Complex architecture with epithelial atypia (resembles well-differentiated adenocarcinoma).
- Approximately, 25–40% patients having complex hyperplasia with atypia develop adenocarcinoma.

2. WHO Classification

Non-atypical Hyperplasia

Increase in number, size and variation in shape of the glands is seen. Even with a back-to-back arrangement of glands, some intervening stroma is visible. This type of hyperplasia results from persistent oestrogenic stimulation and rarely gives rise to endometrial cancer.

Atypical Hyperplasia
Complex architectural patterns with cellular atypia is the hallmark. Atypical hyperplasia is difficult to differentiate from a well-differentiated carcinoma on biopsy. About 20–30% of these cases show foci of endometrial carcinoma on hysterectomy.

Q. Write in detail on the aetiology, clinical features and morphology of endometrial carcinoma.

Ans. Endometrial carcinoma is the most common cancer of female genital tract. It presents with irregular or postmenopausal bleeding and leucorrhoea.

Types (Table 18.1)

- Type 1 (constitutes 80% of all cases; is oestrogen associated).
- Type 2 (less common; not associated with hyperoestrogenaemia).

TABLE 18.1. Differences between Types I and II of endometrial carcinoma

Features	Endometrial carcinoma Type 1	Endometrial carcinoma Type II
Age	55–60 years	65–75 years
Predisposing factors	• Unopposed oestrogen stimulation • Obesity • Hypertension • Diabetes • Nulliparity/infertility • Breast carcinoma	Thin physique
Morphological type	Endometrioid carcinoma (mimics normal endometrial glands)	Serous or clear cell type (mimics subtypes of ovarian carcinoma)
Precursor	Endometrial hyperplasia	Atrophic endometrium Endometrial intraepithelial carcinoma
Molecular genetics	Mutations in PTEN, ARID1A (chromatin regulator), KRAS, β-catenin, p53, PIK3CA, FGF2 (growth factor), CTNNB1 (Wnt signalling) and micro-satellite instability (Flowchart 18.2)	Mutations in P53 and PIK3CA (Flowchart 18.3)
Outcome	Low-grade malignancy; spreads mainly via lymphatics	Aggressive; intraperitoneal and lymphatic spread is common

Proliferative endometrium
↓ *PTEN abnormality*
Hyperplasia without atypia
↓ *MLH1 and KRAS abnormalities*
↓ *Microsatellite instability*
Atypical hyperplasia
↓ *ARID 1A, PIK3CA, CTNNB1 and FGFR2 abnormalities*
Grade 1 endometrioid carcinoma

FLOWCHART 18.2. Evolution of Type I endometrial carcinoma.

Atrophic endometrium
↓ *TP53 mutation*
Serous endometrial intraepithelial carcinoma
↓ *FBXW7, PPP2RIA, CCNE1 abnormalities*
Serous carcinoma

FLOWCHART 18.3. Evolution of Type II endometrial carcinoma.

Gross Morphology

- May be exophytic or infiltrative
- Haemorrhage and necrosis common; may give rise to a shaggy, tan-coloured endometrium

Microscopy

Definitive diagnosis is made only when clear invasion of endometrial stroma or myometrium is seen (differential diagnosis is atypical hyperplasia which does not demonstrate invasion).

Criteria for Stromal Invasion

1. Irregular infiltration by glands inducing stromal fibrosis (**desmoplastic response**)
2. Confluent glands, merging and creating a cribriform pattern with minimal intervening stroma
3. Extensive papillary formations
4. Replacement of stroma by masses of squamous epithelium

Histological Types

Most endometrial carcinomas are **adenocarcinomas.**

Based on the degree of differentiation shown by the tumour, **endometrioid (Type I) endometrial adenocarcinoma** is classified into:

- **Well-differentiated adenocarcinoma** which has a back-to-back arrangement of well-formed glands showing minimal atypia (less than 5% solid growth).
- **Moderately differentiated adenocarcinoma** which shows solid sheets of tumour cells in addition to a glandular pattern (5–50% solid growth).
- **Poorly differentiated adenocarcinoma** which is composed of solid sheets of tumour cells with marked cellular atypia and frequent mitoses; glandular pattern is difficult to find (greater than 50% of the tumour shows a solid pattern).

Type II endometrial carcinomas are most often serous carcinomas.

Q. Describe the clinicopathological features of smooth muscle tumours of uterus.

Ans. Smooth muscle tumours of uterus include

1. **Leiomyoma uterus**
   (a) These are oestrogen-responsive benign tumours (also called fibroids) originating from smooth muscle of uterus that generally present with abnormal bleeding, infertility, bladder compression and increased urinary frequency. Increased frequency of abortions, fetal malpresentation and postpartum haemorrhage may be seen in pregnant women with leiomyomas.
   (b) Common during active reproductive life (incidence of 30–50%); their size may increase during pregnancy. May regress or even calcify after menopause.

***Gross*:**

- Round, firm and grey-white tumours, variable in size with the cut surface showing a whorled pattern.
- Sharply circumscribed and surrounded by compressed out myometrium which forms a pseudocapsule.
- Leiomyomas may show different types of secondary changes, eg, **hyaline degeneration** (due to hyaline change), **red degeneration** (due to venous thrombosis and congestion), **mucinous and cystic degeneration** (liquefaction followed by extreme mucinous degeneration), **ischaemic necrosis, fibrosis** and **calcification** (due to circulatory deprivation and precipitation of calcium salts in the tumour).
- Based on the location, leiomyomas are classified into **subserosal** (beneath the serosa), **submucosal** (beneath the mucosa) or **intramural** (embedded in the myometrium).

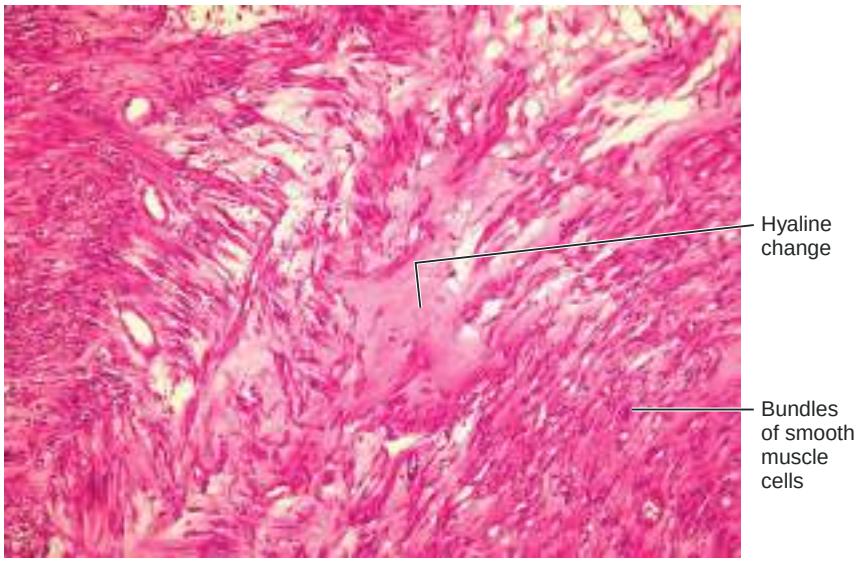


FIGURE 18.3. H&E-stained section from leiomyoma showing intersecting fascicles of smooth muscle cells with hyaline change (H&E; 100X).

- Seventy percent uterine leiomyomas have been found to have mutations in MED12 gene, which encodes for a component of Mediator (a multiprotein complex that forms a bridge between DNA regulatory elements called 'enhancers' and gene promoters).

Microscopy (Fig. 18.3):

- Composed of interlacing fascicles or whorled bundles of smooth muscle cells
- Muscle cells are uniform in size and shape, have oval cigar-shaped nuclei, long bipolar cytoplasmic processes and low mitotic rate.
- Rare variants of leiomyoma include
  - Symplastic or bizarre leiomyoma (cellular tumours with pleomorphic and atypical nuclei but low mitoses)
  - Benign metastasizing leiomyoma (leiomyomas, which may migrate into vessels and other organs, eg, lungs)
  - Disseminated peritoneal leiomyomatosis (manifesting as multiple nodules in the peritoneum)
  - Epithelioid leiomyoma (composed of large epithelioid cells)

2. **Leiomyosarcomas**
   (a) Arise from mesenchymal cells de novo, not from pre-existing leiomyomas.
   (b) Almost always solitary unlike leiomyomas, which are usually multiple.
   (c) May present as bulky masses infiltrating the uterine wall or polypoid masses projecting into the endometrial cavity.
   (d) Show haemorrhage and necrosis.
   (e) Diagnostic histopathologic features are cytological atypia, presence of increased (usually >10mitoses/10HPF) and atypical mitoses and tumour necrosis. In the presence of cytological atypia and epithelioid cells, greater than 5 mitoses/10HPF are enough to label the tumour as malignant.
   (f) Recurrence after removal is common, may metastasize to lungs. Five-year survival is 40%.
3. **Smooth muscle tumours of uncertain malignant potential:**
   Lie at the interface between leiomyomas and leiomyosarcomas and are difficult to classify.

Q. Classify ovarian tumours. Write in detail on their aetiopathogenesis and clinicopathological features.

Ans.

Classification of Ovarian Tumours (WHO)

1. **Surface epithelial-stromal ovarian tumours**
   (a) Occur primarily in adults (second decade onwards).
   (b) Constitute 65–75% of all ovarian tumours.
   (c) Thought to arise by transformation of coelomic epithelium, which may evolve into serous (tubal), endometrioid (endometrial) and mucinous (cervical) epithelium (coelomic epithelium gets incorporated into the ovaries by invagination of the surface epithelium, which later gets detached).

   ***Types*:**
   - **Serous tumours**
     - Benign (cystadenoma, cystadenofibroma)
     - Borderline (serous borderline tumour)
     - Malignant (low- and high-grade serous cystadenocarcinoma)
   - **Mucinous tumours**
     - Benign (cystadenoma, cystadenofibroma)
     - Borderline (mucinous borderline tumour)
     - Malignant (mucinous adenocarcinomas)
   - **Endometrioid tumours**
     - Benign (cystadenoma, cystadenofibroma)
     - Borderline (borderline endometrioid tumour)
     - Malignant (endometrioid adenocarcinoma)
   - **Epithelial–stromal tumours**
     - Adenosarcoma
     - Mixed malignant mesodermal Müllerian tumours (MMMT)
   - **Clear cell tumours**
     - Benign
     - Borderline
     - Malignant
   - **Transitional tumours**
     - Brenner tumour
     - Brenner tumour of borderline malignancy
     - Malignant Brenner tumour
     - Transitional cell carcinoma (non-Brenner type)

2. **Germ cell ovarian tumours**
   (a) Derived from the egg-producing cells within the body of the ovary.
   (b) Occur primarily in children and adolescents.
   (c) Constitute 15–20% of all ovarian tumours and 3–5% of all ovarian cancers.

   ***Types*:**
   - Teratomas
   - Dysgerminomas
   - Endodermal sinus (Yolk sac) tumours
   - Choriocarcinomas
   - Mixed germ cell tumours

3. ***Sex cord–stromal ovarian tumours*:**
   Rare, constitute 2–3% of all malignant ovarian tumours and produce steroid hormones.

   ***Types*:**
   - Granulosa cell tumours
   - Tumours of thecoma-fibroma type
   - Sertoli–Leydig cell tumours
   - Steroid lipid cell tumours

4. ***Cancers derived from other organs (colon, appendix, pancreas, biliary system, breast) can also spread to the ovaries (metastatic cancers).***

Aetiopathogenesis of Ovarian Cancer

- Two most important risk factors are **nulliparity** and **positive family history.**

- Five to ten percent of ovarian carcinomas are familial and may be caused by mutations in BRCA1 and BRCA2 genes (mutations in these genes may increase risk for both ovarian and breast carcinoma).
- The protein HER2/neu is overexpressed in 35% of ovarian cancers and its over-expression is associated with a poor prognosis.
- KRAS is expressed in 30% of tumours (mostly mucinous cystadenocarcinomas).
- p53 is mutated in about 50% of all ovarian cancers.

Low-grade serous adenocarcinomas show KRAS, BRAF or ERBB2 mutations. High-grade tumours show TP53 mutations and lack KRAS or BRAF mutations.

Clinical Features of Ovarian Tumours

- Generally produce no signs or symptoms till they are advanced.
- Clinical presentation is similar despite morphological diversity.
- Functional tumours produce hormones causing endocrinopathies.
- Benign tumours generally produce pressure symptoms due to their size (pain, urinary frequency and gastrointestinal symptoms) and malignant tumours may present with weakness, weight loss and cachexia.
- Torsion of tumours on their pedicles may present as an acute emergency.
- Fibromas and malignant tumours may produce ascites.

Screening Modalities for Ovarian Tumours

- Radiology
- Elevation of markers like glycoprotein CA-125 and osteopontin is noted in 75–90% of women with epithelial ovarian cancers (CA-125 may also be increased, however, in benign conditions as well as nonovarian cancers and may be undetectable in a large number of women with ovarian cancer with no extra ovarian spread).

Salient Features of Different Ovarian Tumours

1. **Epithelial ovarian tumours (surface epithelial tumours)**
   Behaviour of surface epithelial tumours depends on their pathological features:
   - Benign tumours show simple, nonstratified epithelium, with no cytological atypia.
   - Atypical proliferative tumours (borderline tumours) show epithelial proliferation with stratification and tufting, variable mitotic activity and nuclear atypia, but no stromal invasion.
   - Malignant tumours (carcinomas) show stromal invasion and marked cytological atypia.

   ***Types*:**
   - **Serous:** Lining resembles fallopian tube epithelium.
   - **Mucinous:** Lining resembles gastrointestinal tract or endocervical epithelium.
   - **Endometrioid:** Lining resembles proliferative endometrium.
   - **Clear cell:** Lining resembles gestational endometrium.
   - **Transitional cell (Brenner):** Lining resembles urinary tract epithelium.

   (a) Serous tumours
   - Most frequent ovarian tumours.
   - Sixty percent are benign tumours, 15% of low malignant potential and 25% malignant.
   - Twenty percent of the benign and 60% of the malignant tumours are bilateral.
   - Average size is smaller than mucinous tumours.
   - Lining may be smooth or papillary (cauliflower-like masses composed of soft brittle tissue may be seen in malignant tumours). Cysts are filled with clear fluid and lined by cuboidal epithelium with apical mucin.
   - Changes suggestive of malignancy are
     - Predominance of papillary projections and solid areas
     - Presence of haemorrhage and necrosis
     - Invasion of cyst wall
     - Irregular nodular surface due to penetration of the serosal covering by the tumour.

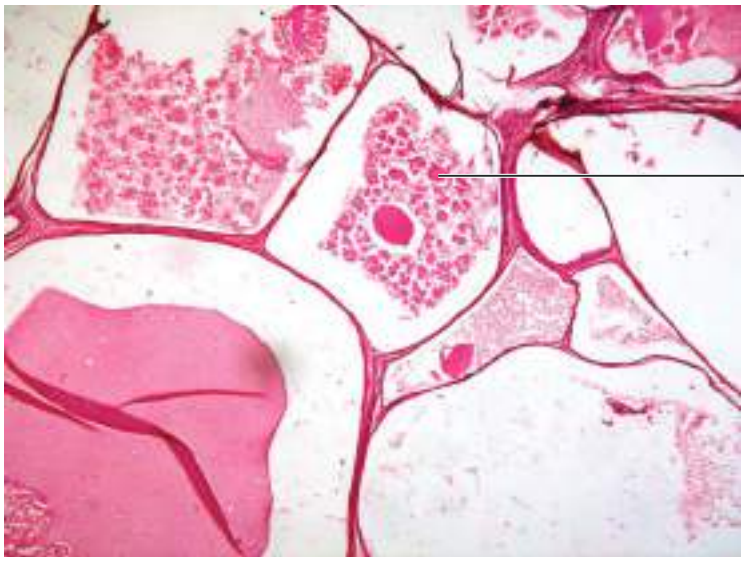


FIGURE 18.4. H&E-stained section from a serous cystadenoma showing multiple cystic spaces lined by cuboidal epithelium with apical mucin (H&E; 100X).

(i) ***Benign serous cystadenoma*:**

***Gross morphology*:**

- Size varies between 15 and 30 cm.
- They are unilocular cystic structures with a smooth glistening wall and contain clear fluid.

Microscopy (Fig. 18.4):

Lining is mostly smooth; may occasionally show papillae which have a central fibrovascular core lined by tall columnar ciliated or nonciliated epithelium.

(ii) ***Borderline (BL) serous cystadenomas*:**

- Constitute 15% of all serous tumours.
- Majority limited to ovary; some show extra-ovarian spread.

***Gross morphology*:** Have a greater papillary component than benign serous cystadenoma.

***Microscopy*:**

- Stratification of the epithelial lining of papillae with formation of microscopic papillary tufts.
- Nuclear atypism and increased mitotic activity may be seen.
- Absence of stromal invasion even on extensive sampling (one block for every 1–2 cm of tumour diameter). Deep invaginations should not be confused with invasion.

(iii) ***Frank serous carcinoma***

- Most common malignant tumour of ovary
- Arises between 45 and 65 years

***Gross morphology*:**

- Average size is 5–15 cm; predominantly solid with variable cystic areas.
- External surface is smooth or papillary; soft friable papillae fill the cavity.

***Microscopy*:**

- Well differentiated: Papillary structures well formed with prominent fibrous stalks.
- Moderately differentiated: Papillae crowded together; individual stalks cannot be discerned.
- Poorly differentiated
  - Papillary pattern obliterated; solid sheets of pleomorphic cells are seen.
  - Prominent mitotic activity
  - Capsular invasion present

- Psammoma bodies in 32% cases (thought to be associated with a better survival)
- Five-year survival <20%

(b) **Mucinous tumours**

- Common in the reproductive age group
- Eighty percent benign, 10% of low malignant potential and 10% are frankly malignant.
- Five percent benign and 20% malignant tumours are bilateral.
- Larger and more multilocular than their serous counterparts.
- Papillary formations and psammoma bodies less common than their serous counterparts.
- Lined by tall columnar epithelium with a basal nucleus and abundant cytoplasmic mucin (cells similar to endocervical mucosa).
- Multiloculated cysts filled with sticky or gelatinous mucinous material.
- Glistening, smooth and papery thin wall.
- Solid areas or papillary projections on inner wall of the cyst suggestive of malignant change.

(i) ***Benign mucinous cystadenoma***

***Gross morphology*:**
Multilocular thin-walled cysts containing sticky gelatinous material.
Microscopy (Fig. 18.5):
Cysts are lined by endocervical or intestinal type of epithelium

(ii) ***Borderline (BL) mucinous malignancy***

***Gross morphology*:** Large, multilocular, cystic, with a smooth external surface; papillary excrescences may be seen.
***Microscopy*:** BL mucinous tumours are similar to BL serous tumours.

(iii) ***Frank mucinous carcinoma***

Constitutes 6–10% of all malignant primary ovarian tumours.
***Gross morphology*:** Cystic and multiloculated with a size up to 50 cm. Commonly shows solid areas, haemorrhage and necrosis.
***Microscopy*:**

- Well-differentiated tumours: Well-defined, gland-like structures or cysts lined by tall columnar mucin-producing cells with few mitoses.
- Moderately differentiated tumours: Few well-defined glands lined by atypical epithelium with numerous mitoses.

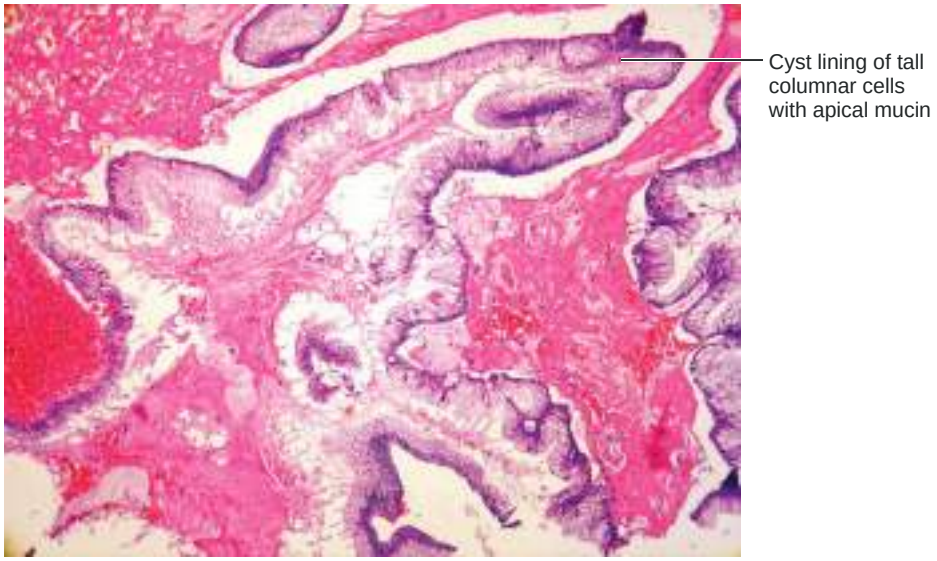


FIGURE 18.5. H&E-stained section showing a mucinous cystadenoma lined by tall columnar epithelium with a basal nucleus and abundant cytoplasmic mucin (cells similar to endocervical mucosa H&E; 100X).

- Poorly differentiated tumours: Irregular nests and cords composed of highly atypical epithelium invading the stroma.

(iv) ***Pseudomyxoma peritonei***
- Seen in 2–5% cases of ovarian mucinous tumours.
- Characterized by massive gelatinous accumulation arranged in a loculated pattern in the peritoneal cavity.
- Large amount of mucin; reaches peritoneal cavity either by dissection through cyst wall or through a perforation.
- Strips of mature cells with basally arranged regular vacuoles filled with mucous or individual epithelial cells found free floating within gelatinous masses.

(c) **Endometrioid ovarian tumours:**
- Ten to twenty-five percent of all surface epithelial tumours.
- Thirty percent are bilateral: 15–30% have a concomitant endometrial carcinoma.
- Solid-cystic; may arise as a mass from an endometriotic cyst filled with chocolate-coloured fluid.
- Lined by tall columnar epithelium with a centrally located nucleus.

(d) **Brenner tumour:** Derived from coelomic epithelium of ovary; forms the typical urothelial-like epithelial elements through a metaplastic process.

(i) ***Benign Brenner:*** Rare ovarian neoplasm; affect individuals more than 50 years.
***Gross morphology*:**
- Usually an incidental discovery (because of a large frequency of microscopic lesions).
- Average size is 2–8 cm; may produce compression of surrounding ovarian tissue.
- Solid, encapsulated, firm and grey-white with a whorled cut surface.

***Microscopy*:**
- Solid-cystic epithelial nests surrounded by a stroma composed of bundles of tightly packed spindle-shaped cells.
- Epithelial cells are polygonal to squamoid with pale eosinophilic cytoplasm and oval nucleus showing longitudinal grooving (coffee-bean appearance).
- No mitotic figures or atypia is seen.
- Association of Brenner with other cystic neoplasms, eg, mucinous cystadenoma and cystic teratoma are well known.

(ii) ***Borderline malignant Brenner tumour***
- Cystic with papillary fronds
- Epithelium resembles noninvasive papillary transitional cell carcinoma of urinary bladder or grade 3 dysplasia (squamous cell carcinoma in situ)

(iii) ***Malignant Brenner tumour*:** Shows frankly malignant histological features with stromal invasion by epithelial elements. Malignant component may be:
- Transitional cell carcinoma.
- Squamous cell carcinoma
- Undifferentiated carcinoma

(e) **Cystadenofibroma:** In some serous neoplasms, fibroblastic stromal component is unduly prominent, appearing as a solid white nodular focus in an otherwise cystic lesion; may be benign, borderline or malignant.

(f) **Clear cell carcinoma** (mesonephroid carcinoma):
***Gross morphology*:** Spongy and cystic.
***Microscopy*:**
- Tubular cystic, papillary or sheet-like arrangement of neoplastic epithelium.
- Tumour cells are large with a clear cytoplasm and nuclei projecting into the lumina (hob nailing).
- Originally thought to originate from mesonephric rests but now definitely known to arise from surface epithelium.

2. **Tumours of germ cell origin**

(a) **Dysgerminoma:**
- Malignant tumour which arises in second to third decades.
- Counterpart of testicular seminoma.
- Associated with gonadal dysgenesis.

- Majority are unilateral, solid large tumours showing sheets and nests of cells with clear cytoplasm and well-defined cytoplasmic margins, separated by thin fibrous strands. Stroma contains lymphoid cells and may show granulomatous inflammation.
- Radio responsive with 80% survival.

(b) **Teratoma:** Constitutes 15–20% of ovarian tumours; more than 90% are benign mature cystic teratomas. Other types include immature, malignant and specialized teratomas.

(i) ***Benign mature cystic teratomas***

- Most common type is a **dermoid cyst** (Fig. 18.6) which is usually cystic; the cyst is lined by stratified squamous epithelium and appendageal structures (ectodermal differentiation) and filled with sebaceous secretion and matted hair.
- They are usually discovered accidentally on radiographs or sonograms, picked up easily due to calcification and teeth formation.
- Ninety percent are unilateral and may present with infertility and torsion (acute surgical emergency).
- Foci of bone and cartilage, bronchial and intestinal epithelium may sometimes be appreciated, indicating development along other germ cell layers.
- Rarely, one of the tissue elements may undergo malignant change, usually a squamous cell carcinoma (when it is referred to as a **teratoma with malignant transformation**).

(ii) ***Immature malignant teratomas***

- Bulky, predominantly solid tumour showing foci of necrosis.
- Immature bone, cartilage, muscle, nerve and other structures are seen on microscopy.
- Also seen are areas of neuroepithelial differentiation (lesions with such areas tend to be aggressive and metastasize widely).

(iii) ***Specialized (monodermal) teratomas***

Teratomas with specialized tissue, eg, **struma ovarii** composed entirely of mature thyroid tissue that may hyperfunction and produce hyperthyroidism or the **ovarian carcinoid,** which can produce carcinoid syndrome.

(c) **Yolk sac (endodermal sinus) tumour of ovary**

- Common in children and young adults
- Usually unilateral and presents with abdominal pain and a rapidly growing pelvic mass

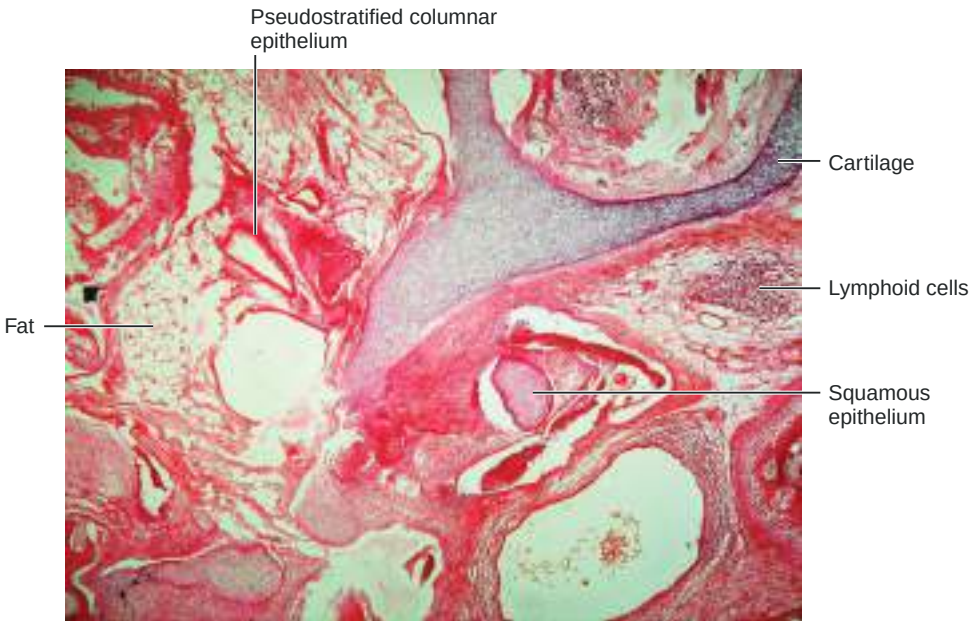


FIGURE 18.6. H&E-stained section from a cystic teratoma showing stratified squamous epithelium and appendageal structures (ectodermal differentiation H&E; 40X).

- Fatal within two years of diagnosis

***Morphology*:** See testicular neoplasms

(d) **Choriocarcinoma**
- Arises in the first three decades of life
- Always unilateral
- Primary focus may disintegrate leaving only metastatic deposits
- Primary may be represented by a small haemorrhagic focus
- Consists of two types of cells, cytotrophoblasts and syncytiotrophoblasts
- Metastasize early and widely

3. **Sex cord tumours**

(a) **Granulosa theca tumours**
- Majority arise in postmenopausal women, but may occur at any age.
- Unilateral small to large, yellow, with cystic spaces.
- Composed of cuboidal granulosa cells in cords, sheets or strands along with spindled or plump lipid-laden theca cells, which elaborate large amounts of oestrogen.
- May recapitulate primitive ovarian follicles called **Call–Exner bodies.**

(b) **Thecoma-fibroma**
- May affect any age
- Unilateral, solid grey with spindled fibrous cells to plump lipid-laden theca cells
- Most are hormonally inactive; few secrete oestrogens
- About 40% produce ascites and hydrothorax **(Meigs syndrome)**
- Rarely malignant

(c) **Sertoli–Leydig cell tumours**
- Affect all ages
- Unilateral, small grey to red brown, solid masculinizing tumours, which recapitulate the development of testes
- Rarely malignant

4. **Metastasis to the ovary**
- Seen in older age group
- Mostly bilateral
- Large, solid, grey-white tumours with cords, glands and individual malignant cells dispersed in the ovarian stroma, eg, **Krukenberg tumour.**

Q. Describe the clinicopathological features of Krukenberg tumour.

Ans. Krukenberg tumour generally affects women more than 45 years.

Pathogenesis

- Most commonly, it is a diffuse type of gastric cancer, which metastasizes to ovaries.
- Two theories have been offered to explain metastasis of tumour from GIT to ovary:
  - Spread due to shedding of cells into peritoneum (transcoelomic spread)
  - Lymphatic spread
- Other cancers associated with Krukenberg tumour are carcinoma of breast, uterus, colon and lung.

Gross Morphology

Bilateral, symmetrically enlarged ovaries, which retain their shape and architecture.

Microscopy

Signet ring cells (cells with abundant mucin, which pushes the nucleus to periphery) are arranged in a diffusely infiltrative growth pattern, that is, cords and singly lying cells with very few glands.

Q. Differentiate between serous and mucinous ovarian tumours.

Ans. Differences between serous and mucinous ovarian tumours are listed in Table 18.2.

TABLE 18.2. Differences between serous and mucinous ovarian tumours

Features	Serous tumours	Mucinous tumours
Frequency	Most common ovarian tumour	Less common than serous tumours
Incidence of malignancy	Account for 60% of all malignant ovarian tumours	Account for 10% of malignant ovarian tumours
Age affected	• Benign lesions: 30–40 years, • Malignant lesions: 45–65 years	Middle age; rare before puberty and after menopause
Bilateralism	Common	Less/rare
Gross	Unilocular/few cysts filled with clear serous fluid	Multilocular tumours filled with sticky gelatinous fluid rich in glycoproteins
Cell lining	Tall columnar ciliated epithelial cells	Tall columnar cells resembling endocervical or intestinal epithelium
Papillae	Very common	Less common
Psammoma bodies	Common	Not found

Q. Differentiate between mature and immature teratoma.

Ans. Differences between mature and immature teratoma are listed in Table 18.3.

TABLE 18.3. Differences between mature and immature teratoma

Features	Mature teratoma	Immature teratoma
Component tissue	Mature	Immature
Age affected	Young women (reproductive age group)	Adolescents and young adults (before age 20)
Bilateralism	Bilateral in 10–15% cases	Mostly unilateral
Type	Mostly cystic (dermoid cyst)	Usually solid
Gross appearance	Unilocular cyst lined by the epidermis. Cyst may have areas of calcification, teeth, matted hair and sebaceous material	Predominantly solid with areas of necrosis and haemorrhage
Microscopy	• Cyst wall lined by mature stratified squamous epithelium with appendageal structures. • No immature elements/neuroepithelium seen	• Immature structures differentiating towards cartilage, glands, muscles, bones, neuroepithelium, etc., seen. Tissue resembles fetal or embryonic tissue rather than adult tissue. • Proportion of immature neuroepithelium in tumour determines the prognosis

Q. Write briefly on gestational trophoblastic disease.

Ans. Gestational trophoblastic disease usually develops within uterus, but may develop at any site of ectopic pregnancy.

- Ranges in behaviour from benign hydatidiform mole (H. mole) to highly aggressive choriocarcinoma.
- All secrete human chorionic gonadotropin (HCG), which can be detected in the serum and urine.
- The fall or rise in titres of HCG can be used as an indicator of response to therapy.

H. Mole

- Traditionally discovered during 12–14 weeks of pregnancy.
- Uterine enlargement is more than what is anticipated for that period of gestation.
- Manifests with vaginal bleeding and passage of grape-like tissue mass.
- Elevation of HCG (particularly the beta subunit) in blood and urine and absence of fetal parts or fetal heart sound on sonography is diagnostic.
- It is of two types, namely, complete and partial mole.

Gross Morphology

- Uterine cavity/ectopic site is filled with delicate, friable masses of thin-walled, translucent, cystic and grape-like structures.
- Amniotic sac is very small and collapsed.
- No fetal parts in complete mole; may be seen in partial mole.

Microscopic Examination

- ***Complete mole***
  - All villi show hydropic swelling and complete loss of vascularity.
  - The central substance of the villi is loose, myxomatous and oedematous, covered by a layer of chorionic epithelium (cytotrophoblast and syncytiotrophoblast).
  - Villi show circumferential proliferation of epithelium to produce sheets and masses of the same.
- ***Partial mole***
  - Villous oedema restricted to some villi.
  - Trophoblastic proliferation is mild and focal.
  - Villi have a characteristic irregular scalloped margin.

Q. Differentiate between partial and complete mole.

Ans. Differences between partial and complete mole are listed in Table 18.4.

TABLE 18.4. Differences between partial and complete mole

Features	Complete mole	Partial mole
Karyotype	46, XX (46, XY)	Triploid (69, XXY)
Incidence of missed abortion	+	+++
Heavy bleeding	+++	+
Toxaemia	+++	+/–
Villous oedema	All chorionic villi are oedematous	Only some villi are oedematous
Trophoblastic proliferation	Diffuse; circumferential	Focal; mild
Vascularization of villi	Absent or inadequate	Present
Fetal parts	No embryonic development, so no fetal parts present	Embryo is viable for weeks, so fetal parts may be present
Atypia	Frequently present	Absent
Serum HCG	Markedly elevated	Elevated, but comparatively less than complete mole
HCG in tissues	++++	+
Behaviour	2% incidence of choriocarcinoma	Incidence of choriocarcinoma negligible

Q. Differentiate between H. mole and choriocarcinoma.

Ans. Differences between H. mole and choriocarcinoma are listed in Table 18.5.

TABLE 18.5. Differences between H. mole and choriocarcinoma

Features	H. mole	Choriocarcinoma
Definition	Variable trophoblastic proliferation, mostly benign in nature. May give rise to choriocarcinoma	Choriocarcinoma is a malignant neoplasm of trophoblastic cells derived from any form of previously normal or abnormal pregnancy
Presentation	During 12–14 weeks of pregnancy, patient presents with vaginal bleeding/passage of grape-like structures	During pregnancy or after miscarriage, patient presents with bloody, brown, foul-smelling discharge
Gross	Delicate, friable mass of thin-walled, translucent, cystic and grape-like structures	Soft, fleshy, with extensive haemorrhage and areas of necrosis
Microscopy	Villous oedema with absence of vascularity, cytotrophoblast and syncytiotrophoblast cover villi	Epithelial malignancy; chorionic villi not formed; abnormal proliferation of both cytotrophoblast and syncytiotrophoblast
Uterus size	Much larger than expected for that duration of pregnancy	Mild enlargement
HCG level	↑	↑↑↑
Precedent history	None	H. mole, abortion, normal/ectopic pregnancy
Metastasis	Not seen	To lung, liver and brain
Treatment	Curettage	Curettage and chemotherapy; may be hysterectomy

19

The Breast

- Breast is a modified apocrine gland, which is composed of 6–10 major ductal systems that can be traced from the nipple (keratinizing stratified squamous epithelium of the skin continues into the ducts and abruptly changes into double-layered cuboidal epithelium).
- The larger ducts branch successively and lead to **terminal duct lobular units (TDLUs;** Fig. 19.1).
- Terminal duct further branches into clusters of small acini to form a lobule.
- Ducts and lobules are lined by two cell types:
  - A basal layer of low, flattened and contractile **myoepithelial cells**
  - A second luminal layer of **epithelial cells**
- Majority of breast stroma consists of dense fibrous connective tissue admixed with adipose tissue **(interlobular stroma)**.
- Present within the lobules is breast specific, hormone responsive and delicate myxomatous stroma **(intralobular stroma)**.

Q. Write briefly on benign epithelial lesions of the breast.

Ans. Benign epithelial lesions of the breast include the various benign alterations in its ducts and lobules. They are classified into three types, depending on the subsequent risk of breast cancer, namely:

- Nonproliferative breast changes (also called fibrocystic changes)
- Proliferative breast disease without atypia
- Atypical hyperplasia

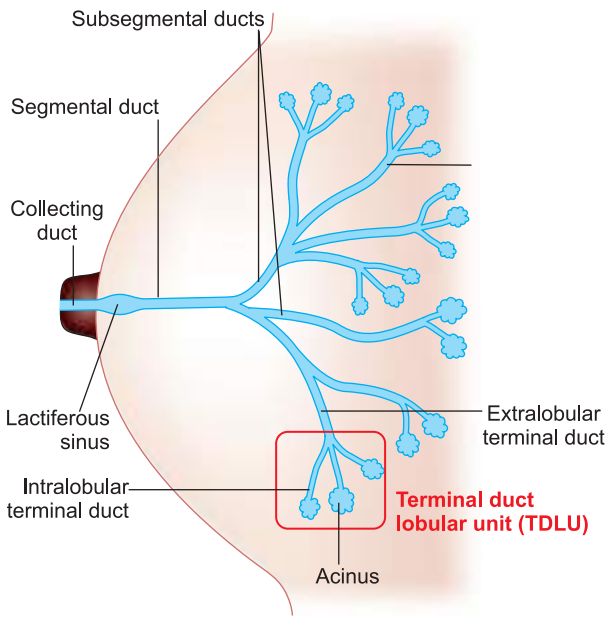


FIGURE 19.1. Diagrammatic representation of normal breast parenchyma.

Nonproliferative Breast Changes

Clinically significant disease (lumpy-bumpy breast) without epithelial hyperplasia

Clinical Features

- Presents with an ill-defined lump/nodularity, mammographic densities/calcification or nipple discharge
- Affects women between 20 and 40 years; peaks at or just before menopause; is rare after menopause and before adolescence
- Usually multiple and bilateral
- No risk of developing cancer
- Clue to diagnosis is disappearance of the mass after fine needle aspiration of cyst contents

Aetiology

Hormonal imbalance: increased oestrogen and decreased progesterone

Morphology

Three main morphological changes are seen:

- Cystic dilation of ducts and lobules
  - Large cysts contain semitranslucent and turbid fluid, which imparts brown to blue colour to them (blue dome cysts)
  - Epithelium lining the cysts is flattened and atrophic; may show apocrine metaplasia (large polygonal cells that have abundant granular eosinophilic cytoplasm and small round hyperchromatic nucleus)
- Fibrosis
  - Cysts release a secretory material into the stroma, which causes chronic inflammation and fibrosis with loss of the normal myxomatous appearance.
- Adenosis
  - Increase in the number of acini per lobule. The acini are lined by columnar epithelium which may occasionally show nuclear atypia (labelled "flat epithelial atypia"—a clonal disorder associated with deletions of chromosome 16q which is thought to be the earliest recognizable precursor of low-grade malignancy; however, does not necessarily translate into an increased risk of invasive breast cancer).
  - The acini are enlarged only (as in blunt duct adenosis) or enlarged and distorted (as in sclerosing adenosis).
  - Calcifications may be seen in the lumens.

Proliferative Breast Disease Without Atypia

Proliferative (hyperplastic) changes may be seen in the ductules, terminal ducts and sometimes the lobules. They are classified into:

(a) Epithelial hyperplasia:
  (i) Defined as presence of more than two cell layers in the lining of ducts and lobules
  (ii) Can vary from mild to florid hyperplasia
  (iii) The ducts, ductules and lobules are filled with cuboidal cells showing small glandular pattern called fenestrations.

(b) Sclerosing adenosis:
  (i) Less common but significant type of proliferative breast disease because of its clinical and morphological similarity to invasive carcinoma.
  (ii) Characterized by marked intralobular fibrosis and proliferation of small ductules and acini.
  (iii) On gross examination, sclerosing adenosis appears hard and rubbery like invasive breast carcinoma.
  (iv) Histopathology sections show proliferation of epithelial and myoepithelial cells lining small ducts and ductules. The proliferating glands and ductules appear back to back. There is marked stromal fibrosis which compresses and distorts the proliferating epi-

thelium leading to obliteration of the lumina of the glands so that they appear as solid cords of cells, closely mimicking an invasive carcinoma). Identification of the myoepithelial cells is an important clue to indicate the benign nature of the lesion.

(c) Complex sclerosing lesion: Complex sclerosing lesion may be a part of sclerosing adenosis, papillomatosis or radial sclerosing lesion (radial sclerosing lesion closely mimics invasive carcinoma, radiologically and pathologically; and is composed of a central nidus of glands entrapped in a hyalinized stroma).

(d) Papillomas: Grow within ducts and are composed of fibrovascular cores lined by luminal and myoepithelial cells; may be large duct papillomas (solitary and located in lactiferous sinuses) or small duct papillomas (multiple and located in the deeper ducts). Usually present with nipple discharge.

Proliferative Breast Disease With Atypia

(a) In some cases, the cells lining ducts show monomorphic hyperplasia and form a regularly spaced pattern (atypical ductal hyperplasia).

(b) Atypical lobular hyperplasia is a term used to describe a hyperplasia that resembles lobular carcinoma in situ, but in which the cells do not fill or distend more than 50% of the acini within a lobule. Atypical lobular hyperplasia is associated with increased risk of invasive carcinoma.

Relationship Between Benign Epithelial Breast Lesions and Invasive Carcinoma

- **Nonproliferative breast disease** includes fibrosis, cystic change, apocrine metaplasia, mild hyperplasia, duct ectasia, adenosis and fibroadenoma; associated with minimal or no increased risk.
- **Slightly increased risk (1.5–2 times)** is noted with moderate or florid hyperplasia without atypia, papillomatosis, sclerosing adenosis, radial sclerosing lesion and fibroadenoma with complex features.
- **Significantly increased risk (4–5 times)** is noted with atypical hyperplasia (ductular or lobular).
- A family history of breast cancer may increase the risk in all categories, eg, atypical hyperplasia (ductular or lobular), associated with a family history may increase the risk ten-fold.

Q. Write briefly on stromal tumours of breast.

Ans. There are two main stromal tumours of breast—**fibroadenoma** and **phyllodes tumour**, both of which arise from the intralobular stroma.

Fibroadenoma Breast (FA)

Salient Features

- It is the commonest benign neoplasm of the female breast, thought to arise as a result of an absolute or relative increase in oestrogen activity.
- A biphasic tumour of stromal origin, it has both the stromal (neoplastic) and epithelial (nonneoplastic) components.
- FA occurs within the reproductive age group with a peak incidence in the third decade, when it presents as a palpable, well-defined, freely mobile mass, which bulges above the breast surface.
- FA is known to regress and calcify after menopause; it sometimes shows features overlapping with fibrocystic disease, when it is labelled **fibroadenomatoid change.**
- FAs almost never become malignant.

Gross Morphology

- Discrete spherical nodule 1–10 cm in diameter (more than 10 cm, labelled as **giant fibroadenoma**).
- Freely mobile, sharply circumscribed and easily enucleated.

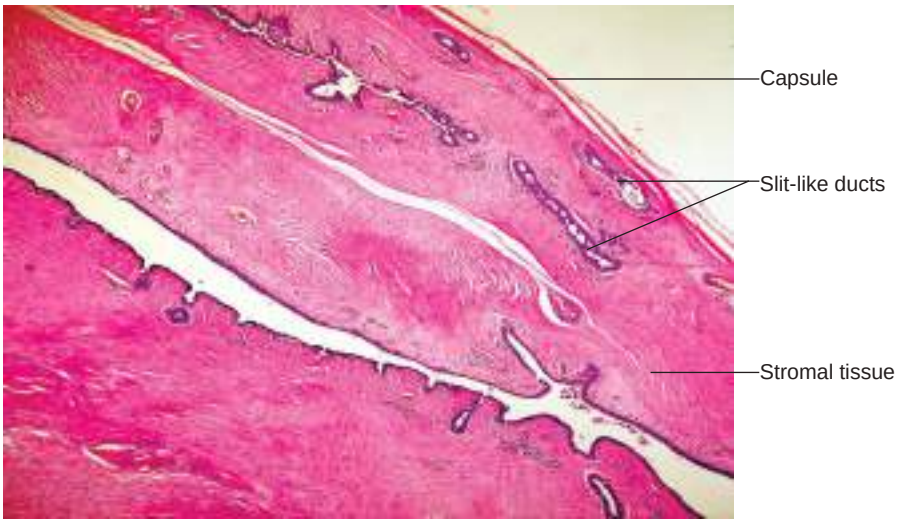


FIGURE 19.2. H&E-stained section from fibroadenoma breast showing a pericanalicular pattern with slit-like ducts surrounded by stromal tissue and enveloped by a well-formed capsule (H&E; 100X).

Microscopic Features

- Stromal overgrowth and ductal proliferation produces two patterns, which may coexist in the same tumour:
- **Intracanalicular pattern:** Delicate myxoid stroma compresses ducts to slit-like spaces lined by ductal epithelium, which appears as cords of epithelium surrounded by abundant fibrous stroma.
- **Pericanalicular pattern:** Abundant stroma surrounds patent or dilated ducts (Fig. 19.2). The stroma may get hyalinized and the lining epithelium may become atrophic in older patients.

Phyllodes Tumour

Salient Features

- 'Phyllodes tumour' is a name given to an uncommon bulky breast tumour with **leaf-like gross appearance.**
- Affects any age, but is more common in the sixth decade, 10–20 years later than fibroadenoma.
- Arises from intralobular, periductal stroma and not from a pre-existing fibroadenoma.
- The term 'cystosarcoma phyllodes' is a misnomer because most of these tumours are benign and without cysts.
- They are associated with acquired clonal chromosomal aberrations like gain in chromosome 1q. High-grade tumours are associated with overexpression of HOXB13.

Gross Morphology

- May be a few centimetres to massive, involving the whole breast.
- Cut surface is grey-white with cystic cavities, areas of haemorrhage and necrosis may be seen.

Microscopic Features

- **Low-grade tumours** resemble fibroadenoma, but cellularity and mitotic figures are increased (Fig. 19.3).
- **High-grade tumours** are like other soft tissue sarcomas; differentiated from low-grade lesions on the basis of cellularity, mitotic rate, nuclear pleomorphism, stromal overgrowth and infiltrative borders.

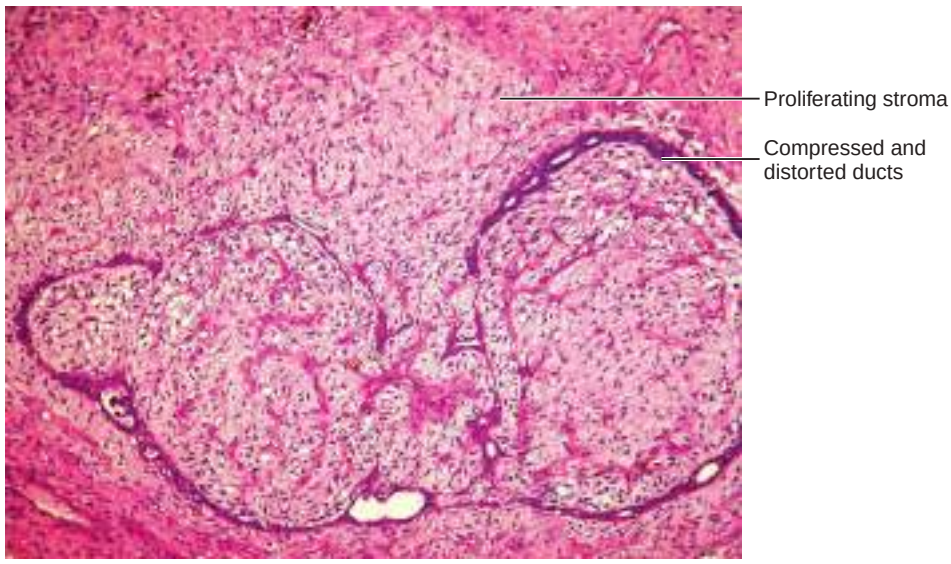


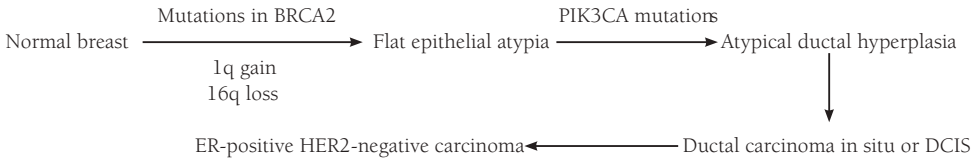
FIGURE 19.3. Low-grade phyllodes tumour showing an exaggerated intracanalicular growth pattern with increased stromal cellularity (H&E; 100X).

Q. Describe the aetiopathogenesis, clinical features and morphology of carcinoma breast.

Ans. All breast carcinomas arise from the terminal duct lobular unit and usually affect women in the third decade onwards.

Classification of Carcinoma Breast

1. **Molecular classification:** Almost all breast carcinomas are adenocarcinomas. They are categorized into three biological groups from the therapeutic perspective:
   (a) Oestrogen receptor (ER)-positive, human epidermal growth factor receptor (HER)-2-negative (50–60% of all tumours) Also called 'luminal A tumours', they are the most common type of breast cancer in patients with germline mutations in BRCA2 (Flowchart 19.1)

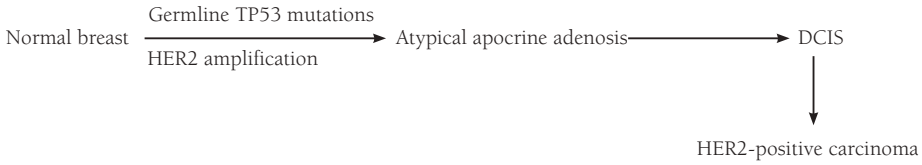


FLOWCHART 19.1. Pathway of development of ER-positive, HER2-negative carcinoma breast.

Salient features:

- ER-positive, HER2-negative tumours are slow growing and respond well to hormonal therapy.
- They are further subdivided into 'low proliferation' (more common) and 'high proliferation' (less common) types.
- The 'low proliferation' type typically affects older women and men and is usually detected on routine mammographic screening. Histological types included in this group are well or moderately differentiated lobular, tubular or mucinous carcinomas.
- The 'high proliferation' group includes poorly differentiated lobular carcinomas and are typically associated with BRCA mutations.

(b) HER2-positive; may be ER-positive or -negative (10–20% tumours)
- Associated with amplification of HER2 gene on chromosome 17 (Flowchart 19.2).



FLOWCHART 19.2. Pathway of development of HER2-positive carcinoma breast.

Salient features:
- Affect young women who are TP53 mutation carriers
- Histologically, some may be apocrine type
- Survival <10 years; ER-negative cancers respond to chemotherapy in >30% cases whereas ER-positive cancers respond to about 15% (triple-positive tumours are generally of higher grade).

(c) ER-negative; HER2-negative (10–20% tumours)
- Arise from a pathway independent of ER-mediated changes (Flowchart 19.3).

FLOWCHART 19.3. Pathway of development of ER-negative, HER2-negative carcinoma breast.

Salient features:
- Affect young women who are TP53 mutation carriers
- Triple-negative; high grade; poorly differentiated; poor prognosis
- Histological types include medullary, adenoid cystic, secretory and metaplastic

2. **Histological classification**

(a) Noninvasive: Lesions that are confined to ducts and lobules and have not penetrated the limiting basement membrane. They can be further classified into
- Ductal carcinoma in situ (DCIS)
- Lobular carcinoma in situ (LCIS)

(b) Invasive: Lesions that have penetrated the limiting basement membrane. These include
- Invasive carcinoma of no special type (NST)
- Invasive lobular carcinoma
- Medullary carcinoma
- Mucinous (colloid) carcinoma
- Tubular carcinoma
- Metaplastic carcinoma
- Inflammatory carcinoma
- Other types

Risk Factors for Carcinoma Breast

- **Age:** Rare before 25 years, except in familial cases, peaks at 70–80 years and then declines in incidence. Menarche at age <11 years increases risk by 20% as compared to menarche at age >14 years.
- **Geography:** Six times higher incidence in developed countries but rising incidence in developing countries.

- **Genetic factors:**
  - Mutations in BRCA1 (familial breast and ovarian cancer), BRCA2 (familial breast and ovarian cancer), p53 (Li-Fraumeni syndrome) and CHEK2 (responsible for 1% of all breast cancers).
  - Family history of breast cancer (affected first-degree relatives who do not carry an established breast cancer gene mutation).
  - Overexpression of HER2/neu proto-oncogene.
  - Amplification of RAS and MYC genes.
- **Breastfeeding:** The longer the duration of breastfeeding, the less the incidence of breast carcinoma.
- **Hormonal influences:** Oestrogen excess (long duration of reproductive life, nulliparity, first child at a late age, increasing age and exogenous oestrogens). Oral contraceptives are not known to be associated with an increased incidence. Oophorectomy decreases the risk by decreasing endogenous oestrogens. Also, drugs like tamoxifen (blocks oestrogen) and aromatase inhibitors (decrease oestrogen synthesis) decrease the risk of ER-positive cancers.
- **Environmental factors:** Radiation exposure, organochlorine pesticides (have oestrogen-like effects) and alcohol intake.
- **Proliferative breast disease/carcinoma of contralateral breast or endometrium** (have several common risk factors).
- **Breast density:** High breast density on mammography has a 4–5 times higher risk of ER-positive and ER-negative cancers.
- **Obesity:** Obese women less than 40 years have anovulatory cycles and lower progesterone levels thereby reducing the risk, whereas, postmenopausal obesity increases the risk attributed to oestrogen synthesis in the fat depots.

Familial breast cancer: Many familial cancers and 80–90% of single gene familial breast cancers are due to two autosomal dominant genes: BRCA1 and BRCA2 (Table 19.1).

TABLE 19.1. Differences between BRCA1- and BRCA2-associated breast cancers

Features	BRCA1	BRCA2
Chromosome	17q21	13q12.3	Smaller
Gene size	Larger		
Functions	• Tumour suppression • Transcription regulation • DNA repair	• Tumour suppression • Transcription regulation • DNA repair	
Age at onset	Younger (40–50 years)	50 years
Risk of other tumours	Carcinoma of ovary (more than BRCA2), prostate, pancreas, fallopian tube and male breast cancer (less than BRCA1).	Carcinoma of ovary, prostate, pancreas, stomach, melanoma, biliary system, pharynx and male breast cancer
Pathology of breast cancers	Greater incidence of medullary carcinoma, poorly differentiated carcinoma, ER-PR and HER2/neu-negative carcinoma and carcinoma with P53 mutations	Similar to sporadic cancers (ER-negative cancers)

Sporadic breast cancer: The main risk factors for sporadic cases of carcinoma breast include oestrogen excess, reproductive history, gender and age.

Distribution of Carcinoma Breast

Central area (20%), upper outer quadrant (50%), upper inner quadrant (10%), lower outer quadrant (10%) and lower inner quadrant (10%). Left breast is more often involved than right.

Pathology of Carcinoma Breast

1. **Noninvasive lesions**
   (a) **DCIS or intraductal carcinoma**
      (i) Most frequently presents as mammographic calcifications; less frequently, as a vaguely palpable mass or nipple discharge. The incidence of DCIS has increased from 5% to 15–30% of all breast carcinomas over the past few years, attributable perhaps to the increasing use of mammographic screening.
      (ii) It may be an incidental finding on biopsy.
      (iii) Consists of a malignant population of cells limited to the ducts by basement membrane.
      (iv) Myoepithelial cells are preserved though may be decreased in number.
      (v) Clonal proliferation of cells usually involving a single ductal system.
      (vi) Has two main architectural subtypes:
         - **Comedocarcinoma**
            - Characterized by solid sheets of pleomorphic cells with central necrosis.
            - Necrotic cell membranes frequently calcify and are seen on mammography as speckled microcalcifications, which may be grouped together or arranged in parallel lines.
            - Periductal concentric fibrosis and inflammation is common.
            - Extensive lesions may be palpable as vague nodularity.
         - **Noncomedo DCIS**
            - Does not show cellular pleomorphism or central necrosis.
            - May show different architectural patterns.
            - Consist of a monomorphic population of cells completely filling up the duct lumina **(solid type)**, cells may grow into the spaces lining fibrovascular cores **(papillary DCIS)**, or project into the spaces without definite fibrovascular cores forming complex intraductal patterns **(micropapillary DCIS)**. **Cribriform DCIS** has a cribriform pattern with round spaces between cell aggregates.
   (b) **LCIS**
      (i) Usually, an incidental finding in breast biopsies performed for some other reason.
      (ii) Not associated with a clinically apparent mass or a mammographic abnormality (calcification or stromal reaction); so not readily diagnosed.
      (iii) Bilateral in up to 40% of the patients when both breasts are biopsied.
      (iv) LCIS is an intraepithelial proliferation of the TDLU. The cells of atypical lobular hyperplasia, LCIS and invasive lobular carcinoma are identical, ie, loosely cohesive, small with oval-to-round nuclei and small nucleoli. LCIS is diagnosed when the entire lobular unit is replaced by tumour cells.
      (v) Signet ring cells containing mucin are frequently seen.
2. **Invasive carcinomas**
   - The terminology for the most common type of breast cancer has changed from invasive ductal carcinoma, not otherwise specified (NOS; 2003) to invasive carcinoma of no special type (NST; 2012). This group of breast cancers comprises all tumours without the specific differentiating features that characterize the other specific categories of breast cancers. The name 'ductal' has been omitted as it indicates derivation of the tumours from only the ductal system. The use of 'carcinoma of no special type' is the preferred term. The diagnosis is made by exclusion of recognized specific types of breast cancers. Other types of breast cancer with specific features are regarded as invasive ductal carcinomas, albeit of special type.
   - The most common specific subtypes include invasive lobular, tubular, cribriform, metaplastic, apocrine, mucinous, papillary and micropapillary carcinoma, as well as carcinoma with medullary, neuroendocrine and salivary gland/skin adnexal type features. These specific tumour types are defined by their morphology, but these are also linked to particular clinical, epidemiological and molecular features.

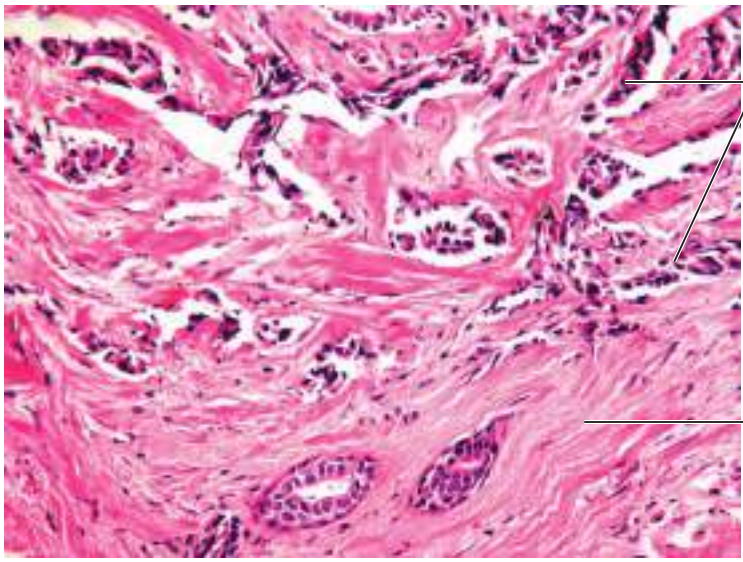


FIGURE 19.4. H&E-stained section from invasive carcinoma breast (NST) showing tubules and cords of pleomorphic cells invading the fibrous stroma (H&E; 200X).

Invasive (infiltrating) carcinoma; no special type (NST)

- Most common type; usually has abundant fibrous stroma (therefore referred to as **scirrhous carcinoma).** It presents as a firm-to-hard lesion which makes a grating sound on cutting.
- It has irregular infiltrating borders with small pinpoint foci or streaks of **chalky-white elastosis/calcification** in the centre of the lesion.
- Well-differentiated tumours consist of tubules lined by minimally atypical cells which express hormone receptors and do not overexpress HER2/neu.
- Less-differentiated lesions are composed of cords and sheets of pleomorphic cells that do not express hormone receptors or overexpress HER2/neu (Fig. 19.4).
- May be accompanied by **variable amounts of DCIS.** Grade of DCIS correlates with the grade of IDC (NOS). Large amounts of DCIS warrants wider excision.

Special subtypes of invasive breast carcinoma

(a) **Invasive lobular carcinoma**

(i) Most cases present as a palpable ill-defined thickening/mass or a mammographic density.

(ii) It is the most common type of breast cancer to present as an occult primary.

(iii) It is associated with a bi-allelic loss of expression of CDH1 (gene encoding for E-cadherin). Loss of E-cadherin induces a discohesiveness in the tumour due to which the tumour is seen histopathologically as single files of tumour cells infiltrating the stroma without induction of a desmoplastic response

(iv) Tumour cells show minimal pleomorphism except in some variants (pleomorphic variant) and appear deceptively monomorphic.

(v) Variants such as solid, alveolar, pleomorphic, tubulolobular and mixed type are recognized and have differences in prognosis when compared to ILC of classic type. Among pleomorphic lobular carcinomas, apocrine, histiocytic or signet-ring cell differentiation can be observed.

(vi) Tumour grading of ILC is advocated, with the majority of classic ILCs being grade 2 in the Nottingham histological grading system and ILC of grade 3 comprising mostly a solid and pleomorphic subtype.

(vii) Immunostaining with E-cadherin can help in distinguishing ILC from NST carcinomas.

(viii) Lobular carcinomas metastasize to unusual sites. Metastasis to meninges, serosal surfaces, retroperitoneum, ovaries and GIT is more common than lungs and pleura.

(b) **Medullary carcinoma**
  (i) Though germline BRCA1 mutations are not present in most of these, hypermethylation of the BRCA1 promoter leading to downregulation of BRCA1 expression is noted in 67% tumours.
  (ii) They presents as well-circumscribed, soft, fleshy masses (*medulla* in Latin means *marrow*), which may be confused with benign lesions.
  (iii) Histopathology shows:
    - A solid syncytial arrangement occupying more than 75% of the tumour, with the tumour cells being large, pleomorphic, having vesicular nuclei with prominent nucleoli with frequent mitoses
    - Lymphoplasmacytic infiltrate surrounding and within the tumour nests
    - Pushing and noninfiltrative tumour margins
    - Minimal or absent DCIS
    - Absence of lymphatic or vascular invasion. Lymph node involvement is rare.
    - A better prognosis than NST.

  The current WHO classification recommends medullary carcinomas and carcinomas with similar features into a group termed 'carcinomas with medullary features'.

(c) **Mucinous (colloid) carcinoma**
  (i) Commonly presents as a circumscribed mass in older women and progresses slowly
  (ii) Soft in consistency with a pale grey-blue gelatinous appearance (due to mucin)
  (iii) Histopathology shows large pools of mucin, scattered within which are small clusters of malignant cells.

(d) **Tubular carcinoma**
  (i) Incidence of this tumour has increased after initiation of mammographic screening.
  (ii) Affects women in their late forties.
  (iii) Tumours are frequently multifocal and bilateral.
  (iv) Histopathology shows well-formed tubules lined by malignant cells. There is absence of myoepithelial cells. Tubular pattern should be seen in more than 75% of the tumour.
  (v) Apocrine snouts are present and calcification is common.
  (vi) Axillary metastasis is seen in fewer than 10% of the cases (excellent prognosis).

(e) **Invasive papillary carcinomas**
  (i) A rare invasive carcinoma with papillary architecture.
  (ii) Clinical presentation is similar to NST but prognosis is better.

(f) **Metaplastic carcinoma**
  (i) Represents a group of invasive breast cancers showing differentiation of the tumour cells into squamous and mesenchymal elements (spindle, chondroid, osseous and rhabdomyoid cells) which are mixed with carcinoma of usual type.
  (ii) Based on nuclear features, metaplastic carcinomas are classified into 'low-grade tumours' (eg, low-grade adenosquamous carcinoma or low-grade spindle cell carcinoma), or 'high-grade tumours' (eg, high-grade squamous cell carcinoma, or high-grade spindle cell carcinoma).
  (iii) They are triple-negative tumours, but have a worse prognosis than other forms of triple-negative breast cancers.

Prognostic or Predictive Factors of Carcinoma Breast

Major prognostic factors

- **Lymph node metastases**: Axillary lymph node status is the single most important prognostic factor. With no involvement, 10-year disease-free survival rate is 70–80%, with 1–3 positive nodes; it is 35–40%, with more than 10 positive nodes, it is 10–15%.

Size of metastatic deposits and **presence of invasion through the capsule** indicates poor prognosis.

- **Locally advanced disease**: Invasion into the skin and skeletal muscle indicates poor prognosis.
- **Inflammatory carcinoma:** Women presenting with a malignant breast mass with redness, oozing, inflamed appearance and skin thickening have a poor prognosis.
- **Tumour size:** Second most important independent factor. Five-year survival rate for tumour of size <1 cm (node-negative) is nearly 98% and it drops to 77% for tumours >2 cm.
- **Distant metastasis:** Presence of distant metastasis indicates poor prognosis.
- **Invasive carcinoma versus in situ disease:** In situ carcinoma is confined to the ductal system and does not metastasize whereas at least half the invasive carcinomas metastasize.

Minor prognostic factors

- **Histological subtypes:** Special types of invasive carcinoma (tubular, colloid, medullary, lobular and papillary) have better prognosis than no special type. Tubular and colloid carcinomas have an exceptionally good prognosis.
- **Tumour grade:** Most commonly used grading system is the **Nottingham Histological Score** or **Scarff Bloom Richardson** grading based on nuclear grade, tubule formation and mitotic rate. Ten-year survival for grade I tumours is 85%; grade II is 60% and grade III is 15%.
- **Oestrogen and progesterone receptors:** Eighty percent of tumours that are both ER- and PR-positive respond to hormonal therapy. Only 40% of those positive only for ER or PR receptors respond to the same. Strongly ER-positive tumours do not respond well to chemotherapy, and tumours that are neither ER- nor PR-positive are more likely to respond to chemotherapy than hormonal therapy.
- **HER2/neu (erb B2):** Over-expression is associated with a bad prognosis. **Herceptin** is a monoclonal antibody to HER2/neu which targets tumour cells (**targeted therapy**).
- **Lymphovascular invasion**: Associated with a poor prognosis.
- **Proliferative rate:** Tumours with high proliferation rates have a worse prognosis.
- **Response to neoadjuvant therapy:** The degree to which the tumour responds to therapy given before surgery is an important prognostic factor. Clinical and radiological examination can be used to assess this response.

The major prognostic factors are used by the American Joint Committee on Cancer, to divide breast carcinoma into the following stages:

- **Stage 0:** DCIS or LCIS (5-year survival rate, 92%)
- **Stage 1:** Invasive carcinoma 2 cm or less in diameter (including carcinoma in situ with microinvasion) without nodal involvement (5-year survival, 87%)
- **Stage 2:** Invasive carcinoma 5 cm or less in diameter with up to three involved axillary lymph nodes.
  Or Invasive carcinoma more than 5 cm without nodal involvement (5-year survival, 75%)
- **Stage 3:** Invasive carcinoma 5 cm or less with four or more involved axillary lymph nodes
  Or Invasive carcinoma more than 5 cm with nodal involvement
  Or Invasive carcinoma with 10 or more involved axillary lymph nodes
  Or Invasive carcinoma with involvement of ipsilateral internal mammary lymph nodes
  Or Invasive carcinoma with skin involvement (oedema, ulceration or satellite skin nodules)
  Or Chest wall fixation or clinical inflammatory carcinoma (5-year survival, 46%)
- **Stage 4:** Any breast carcinoma with distant metastasis (5-year survival, 5–13%)

Q. Write briefly on Paget disease of breast.

Ans. Paget disease of breast is a rare form of DCIS with an incidence of 1–4%.
It presents as an erythematous eruption with scaling and crusting and may be mistaken for eczema.

Pathogenesis

Tumour cells from underlying ductal carcinoma migrate up into the lactiferous duct and invade the epidermis producing a skin lesion without invading the basement membrane.

Gross Morphology

- Skin of nipple and areola is fissured, ulcerated with or without nipple discharge.
- Inflammatory oedema and hyperaemia are seen in the surrounding tissue.
- Underlying palpable mass is present in 50–60% cases of Paget disease.

Microscopic Features

- **Histological hallmark** is involvement of epidermis by malignant cells **(Paget cells).**
- Paget cells are large with abundant clear or lightly stained cytoplasm and nuclei with prominent nucleoli.
- Cells contain mucin and are positive for epithelial membrane antigens (EMA), *c-erb-B2* and low molecular weight keratins.

Prognosis depends on the extent of underlying carcinoma.

MALE BREAST

Q. Write briefly on gynaecomastia.

Ans. Gynaecomastia is defined as enlargement of the male breast. It may be unilateral or bilateral and has the following salient features:

- Presents mostly as a subareolar swelling.
- Causes include idiopathic, hyperestrinism (cirrhosis and functioning testicular tumours), drugs like anabolic steroids, cimetidine, omeprazole, antipsychotics and Klinefelter syndrome.
- Morphological features include proliferation of dense collagenous connective tissue and marked hyperplasia of the ductal and myoepithelial cells. Lobule formation is rare.
- Proliferation of dense collagenous connective tissue.
- Marked hyperplasia of the ductal and myoepithelial cells.
- Lobule formation is rare.

Q. Write briefly on male breast cancer.

Ans. Salient features of male breast cancer

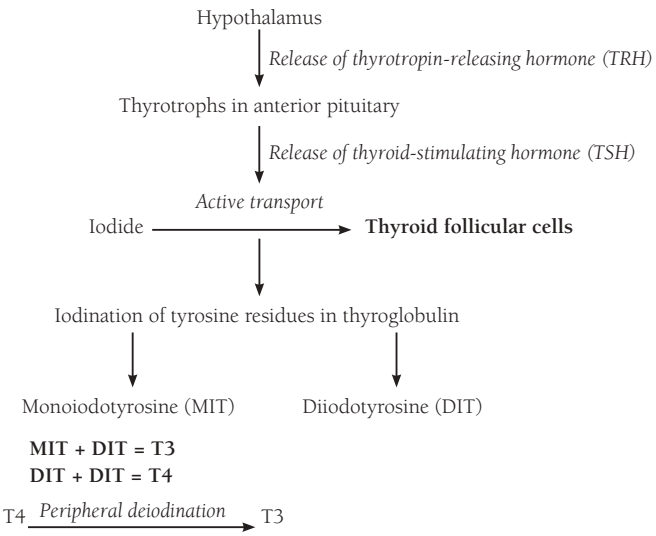
- Incidence is 1% that of females.
- Risk factors are similar to those in women; 3–8% associated with Klinefelter syndrome and decreased testicular function.
- Usually present between sixth and seventh decades with a subareolar mass and nipple discharge (breast epithelium in males restricted to large ducts near areola.
- Associated with BRCA2 and BRCA1 mutations.
- Eighty-one percent are ER-positive; prognostic factors and pathology are similar to female breast cancer.

20

Endocrinology

THYROID

- Weighs 15–20 g
- Has a rich intraglandular capillary network (from superior and inferior thyroid arteries) and nerve supply from cervical sympathetic ganglia
- The gland is divided by thin fibrous septae into lobules composed of 20–40 evenly dispersed follicles.
- Follicles are lined by cuboidal to low-columnar epithelium and contain thyroglobulin.
- Homoeostasis in the hypothalamus–pituitary–thyroid axis ensures maintenance of normal thyroid functioning (Flowchart 20.1).



Free T4 = 0.03% of total T4
Free T3 = 0.3% of total T3
70% of T4 circulates in the peripheral blood bound to **TBG (thyroid-binding globulin)**, **20%** circulates bound to **TBPA (thyroid-binding proalbumin) and 10%** to **TBA (thyroid-binding albumin).** Most T3 is bound to **TBG.**

FLOWCHART 20.1. Mechanism of homoeostasis in the hypothalamus–pituitary–thyroid axis and release of thyroid hormones.

Q. Define thyrotoxicosis. Enumerate the disorders associated with hyperthyroidism.

Ans. Thyrotoxicosis is a hypermetabolic systemic state which occurs due to increased free T3 and T4 levels. It is most commonly caused by hyperfunctioning of the thyroid gland; also known as 'hyperthyroidism'.

Disorders Associated With Hyperthyroidism

Common

- Diffuse toxic hyperplasia **(Graves disease)**
- Toxic multinodular goitre (**Plummer disease**)
- Toxic adenoma

Uncommon

- Acute or subacute thyroiditis
- Hyperfunctioning thyroid carcinoma
- Choriocarcinoma or hydatidiform mole (due to mild thyrotropic effect of HCG)
- TSH-secreting pituitary adenoma
- Neonatal thyrotoxicosis with maternal Graves disease
- Struma ovarii
- Iodide-induced hyperthyroidism
- Iatrogenic (**Job–Basedows disease**)

Q. Write briefly on the clinical manifestations and diagnosis of hyperthyroidism.

Ans. Hyperthyroidism is a systemic state in which there is hyperfunctioning of thyroid gland.

Clinical Manifestations of Hyperthyroidism

- Increased BMR (basal metabolic rate), tachycardia, cardiomegaly, arrhythmias and congestive heart failure (due to increased cardiac contractility) and thyrotoxic dilated cardiomyopathy (shows lymphoeosinophilic infiltration of myocardium with fatty change and fibrosis)
- Generalized lymphoid hyperplasia and lymphadenopathy
- Ocular changes, eg, a wide, staring gaze and lid lag (due to sympathetic overstimulation) and true thyroid ophthalmopathy (as seen in Graves disease)
- Increased appetite, but weight loss; increased gut motility, tremors, hyperactivity, emotional liability, anxiety, inability to concentrate, insomnia and heat intolerance (due to overactivity of sympathetic nervous system)
- Proximal muscle weakness (caused by atrophy and fatty infiltration of skeletal muscle; also called thyroid myopathy)
- Warm and moist skin showing flushing and increased sweating (due to peripheral vasodilatation)
- Bone resorption (causing osteoporosis and increased risk of fractures)
- Fatty liver

Diagnosis of Hyperthyroidism

Hyperthyroidism is diagnosed based on findings of a low serum TSH and increased free T4.

Q. Describe the etiopathogenesis and clinicopathological features of Graves disease.

Ans. Graves disease is the most common cause of endogenous hyperthyroidism with a peak incidence between 20–40 years and a female:male ratio of 7:1. Its genetic basis is

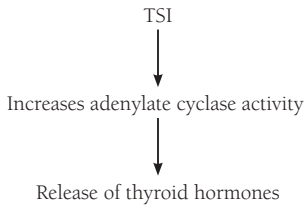
supported by the fact that there is a 60% concordance in monozygotic twins and an association with HLA B8 and DR3. The genetic susceptibility is linked to polymorphisms in multiple immune regulatory genes, eg, cytotoxic T-lymphocyte-associated antigen 4 (CTLA 4) and protein tyrosine phosphatase 22 (PTPN 22). Graves disease is a triad of:

- Hyperthyroidism due to diffuse hyperplasia of follicular epithelium
- Infiltrative ophthalmopathy with resultant exophthalmos
- Localized infiltrative dermopathy called pretibial myxoedema

Pathogenesis

Multiple autoantibodies have been demonstrated in Graves disease, primarily against the TSH receptor. These include:

1. **Thyroid-stimulating immunoglobulin or TSI**
   - TSI is an IgG immunoglobulin that binds to TSH receptor on the membrane of follicular cells and mimics the action of TSH (Flowchart 20.2)
   - Almost all patients demonstrate this antibody
   - It is specific for Graves disease



FLOWCHART 20.2. Mechanism of action of TSI.

2. **Thyroid growth stimulating immunoglobulin or TGI**
   - Also directed against TSH receptor
   - Induces proliferation of thyroid follicular epithelium leading to diffuse hyperplasia of the gland
3. **Thyroid binding inhibitor immunoglobulin or TBII**
   - Also called anti-TSH receptor antibody; it prevents TSH from binding to its receptor on follicular cells.
   - Some forms of TBII mimic the action of TSH causing hyperthyroidism and others actually inhibit thyroid function leading to hypothyroidism.

Triggers for initiation of autoimmune reaction are

- Molecular mimicry
- Primary T-cell autoimmunity

Clinical Features

- Thyrotoxicosis
- Diffuse hyperplasia of thyroid
- Ophthalmopathy
- Dermopathy

} Features unique to Graves disease

Ophthalmopathy

- There is abnormal protrusion of the eyeball (**exophthalmos**), **a wide staring gaze** and **lid lag** (both due to sympathetic overactivity).
- Volume of retro-orbital connective tissue and extraocular muscles is increased due to:
  - Inflammation (abundant $CD4^+$ and $CD8^+$ T cells in the inflammatory population)
  - Accumulation of extracellular matrix components (proteoglycans and hyaluronic acid)
  - Fatty infiltration

Dermopathy
- Skin overlying shins show scaly thickening and induration (**pretibial myxoedema**).
- Also seen are pigmented papules or nodules with orange peel texture.

Laboratory Findings

- Elevated free T3, T4 and TSH
- Increased diffuse radioactive iodine uptake

Gross Morphology

- Diffusely enlarged gland weighing more than 80 g
- Gland is smooth and soft with an intact capsule.
- Cut surface shows a **soft meaty appearance (resembling muscle).**

Microscopy

- Follicles are lined by tall columnar cells showing crowding ('too many cells'), and have pale scalloped colloid.
- Hyperplasia of the follicular lining epithelium results in the formation of hyperplastic papillae or pseudopapillae (papillae without fibrovascular cores).
- Large reactive lymphoid follicles with germinal centres may be present in the interfollicular stroma.

Q. Write briefly on the aetiopathogenesis, clinical manifestations and diagnosis of hypothyroidism.

Ans. Hypothyroidism is a structural or functional derangement that interferes with the production of adequate level of thyroid hormones.

Causes

Thyroidal
- Insufficient thyroid parenchyma:
  - Developmental (thyroid dysgenesis)
  - Radiation injury
  - Surgical ablation
  - Hashimoto thyroiditis
- Interference with thyroid hormone synthesis:
  - Idiopathic primary hypothyroidism
  - Heritable biosynthetic defects
  - Iodine deficiency
  - Drugs (lithium, iodides, P-amino salicylic acid)
  - Hashimoto thyroiditis

Suprathyroidal
- Pituitary lesions (tumours, radiation damage and surgical removal) reducing TSH
- Hypothalamic lesions that reduce thyrotropin-releasing hormone delivery

Classical Clinical Manifestations

- **Cretinism:** Hypothyroidism developing in infancy and childhood which is characterized by impaired development of the skeletal system and CNS presenting as delayed milestones, delayed bone maturation, severe mental retardation, short stature, coarse facial features, protruding tongue and umbilical hernia.

- **Myxoedema:** Hypothyroidism developing in older children or adults characterized by
  - Decreased physical and mental activity, fatigue, apathy, mental sluggishness and depression
  - Slow speech and intellectual functions
  - Increased weight and cold intolerance
  - Reduced cardiac output causing shortness of breath and decreased exercise capacity
  - Constipation and decreased sweating
  - Oedema, broadening and coarsening of facial features, enlargement of tongue and deepening of voice

Laboratory Findings

- Increased TSH and decreased T3 and T4
- Low free T4 and high TSH levels are used for screening

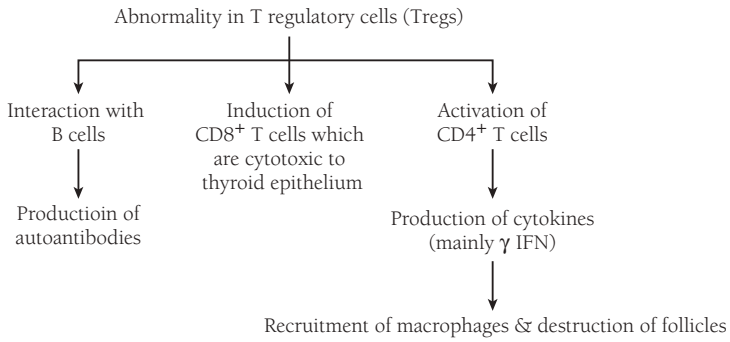
Q. Define and classify thyroiditis. Describe the aetiopathogenesis, clinical features and morphology of the different types of thyroiditis.

Ans. Thyroiditis is inflammation of thyroid gland.

Types

1. **Infectious thyroiditis:**
   - May be acute or chronic
   - Infection reaches thyroid by haematogenous route or through direct seeding of the gland
   - Common causative organisms include mycobacteria, fungi and pneumocystis
2. "**Other common and clinically significant thyroiditis", which include**
   (a) Hashimoto thyroiditis:
   ***Salient features*:**
   - Most common cause of autoimmune thyroiditis
   - May occur in children and is the main cause of nonendemic goitre in this age group.
   - Peak incidence between 45 and 65 years; female:male ratio = 10:1.
   - Clusters in families
   - Concordance in monozygotic twins is 30–60%.
   - Association with HLA-DR3 and -DR5 and increased incidence of SLE, Sjögren syndrome, pernicious anaemia, Type I DM and rheumatoid arthritis in this group.
   - Patients present with painless enlargement of thyroid. There is insidious onset of hypothyroidism after a transient phase of Hashitoxicosis (thyrotoxicosis is due to inflammatory disruption of thyroid follicles leading to the release of thyroid hormones).

   ***Pathogenesis*:**
   - The genetic susceptibility is linked to polymorphisms in multiple immune regulatory genes, eg, CTLA 4 and PTPN 22.
   - Both cellular and humoral mechanisms are involved.
   - Cellular immunity is primarily mediated by a defect in T cells (abnormalities of Tregs or regulatory T cells; exposure of normally sequestrated thyroid antigens; decreased number of suppressor T cells; emergence of thyroid-specific helper T cells, all contributing to autoimmunity).
   - Abnormality in Tregs and breakdown of tolerance leading to autoimmunity (Flowchart 20.3):



B cells produce autoantibodies to:

– **Thyroglobulin and thyroid peroxidase**
  - Thyroglobulin: Follicular cells synthesize thyroglobulin, which is secreted into the lumen as colloid.
  - Thyroid peroxidase: Thyroid peroxidase is located on the luminal surface of follicular cells; catalyses both tyrosine iodination and coupling of iodotyrosyl residues to form T3 and T4. Antibodies to thyroglobulin and thyroid peroxidase are nonspecific.
– **TSH receptor:** TSH receptor is a G protein-coupled transmembrane receptor, antibodies against which are specific in nature.
– **Iodine transporter:** Mediates the transport of iodine into thyroid (first step in thyroid hormone synthesis).

Note: 'Most antithyroid antibodies can fix complement'. Follicular destruction is attributed to complement-dependent, antibody-mediated cytotoxicity (ADCC). Apoptosis by Fas–Fas ligand system is also implicated in destruction of thyroid tissue.

FLOWCHART 20.3. Pathogenesis of Hashimoto thyroiditis.

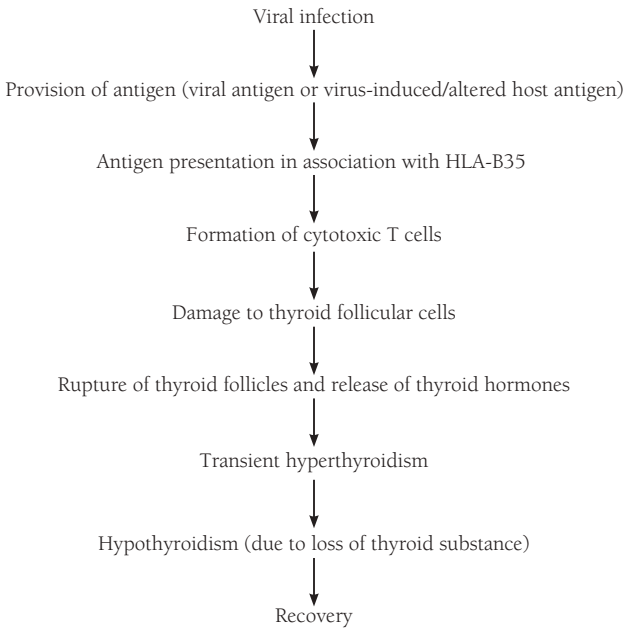
***Gross morphology*:**
- Diffuse/rarely localized enlargement of thyroid
- Capsule remains intact
- Cut surface is pale, grey-tan, firm and rubbery with accentuation of lobulation.

***Microscopy*:**
- Extensive infiltration of parenchyma by a mononuclear infiltrate (lymphocytes, including well-developed germinal centres and plasma cells)
- Atrophy of follicles with presence of **Hürthle cells** (degenerated follicular cells with abundant granular eosinophilic cytoplasm and prominent nucleoli; also called **Askanazy cells** or **oncocytes**)
- Increased interstitial connective tissue; however, fibrosis does not extend outside the capsule.
- Hashimoto thyroiditis has a **fibrous variant,** in which the thyroid becomes small and atrophic due to extensive fibrosis.

(b) Granulomatous thyroiditis/de Quervain thyroiditis

***Salient features*:**
- Peak age 30–50 years; female:male ratio = 3–5:1
- Association with HLA-B35
- Seasonal peak in summers
- Usually follows an upper respiratory tract infection with coxsackie, mumps, measles and adenovirus
- Presents with pain in upper neck, jaw, throat, ears, fever, fatigue, malaise, anorexia, myalgias and enlargement of the thyroid.
- The usual sequence of events is a transient hyperthyroidism (lasting approximately 2–6 weeks) followed by hypothyroidism (lasting 2–8 weeks) followed by recovery.

Pathogenesis (Flowchart 20.4)



FLOWCHART 20.4. Pathogenesis of de Quervain thyroiditis.

***Gross morphology*:**
- Unilateral or bilateral enlargement
- Capsule is intact and may be adherent to surroundings
- Cut surface is yellow-white, rubbery and firm

***Microscopy*:**
Early changes:
- Scattered disruption of follicles
- Replacement by neutrophilic microabscesses

Late changes:
- Aggregates of lymphocytes, histiocytes and plasma cells
- Presence of multinucleate giant cells around pools of colloid
- Fibrosis

(c) Subacute lymphocytic (painless) thyroiditis

***Salient features*:**
- Affects middle-aged women generally in the postpartum period
- Associated with HLA-DR3 and -DR5
- Thought to be variant of Hashimoto thyroiditis
- Patients demonstrate increased levels of antibodies to thyroglobulin and thyroid peroxidase
- Manifests with hyperthyroidism followed by reversion to a euthyroid state. In a minority of patients, the disease may progress to hypothyroidism.

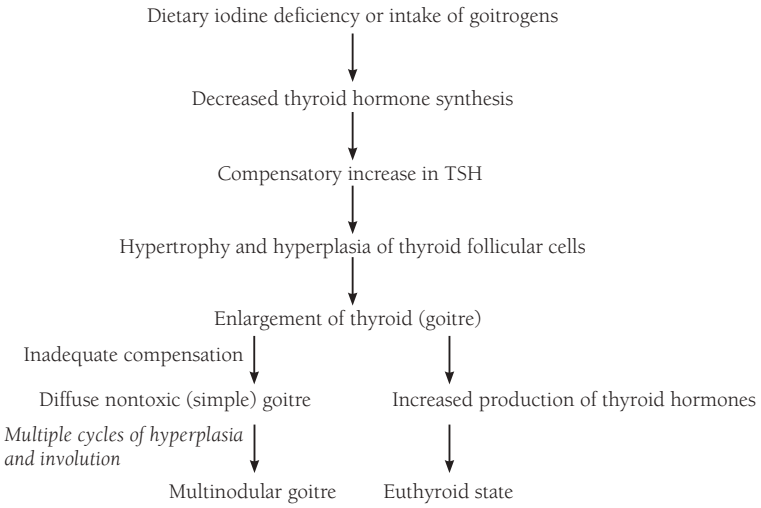
***Gross morphology*:** Mild symmetric enlargement of thyroid

***Microscopy*:**
- Focal disruption of thyroid follicles
- Multifocal inflammatory infiltrate (predominantly small lymphocytes)
- No plasma cells, germinal centres or Hürthle cell metaplasia. If present, think of Hashimoto thyroiditis.

Q. Define and classify goitre. Describe the aetiopathogenesis, clinical manifestations and morphology of the various types of goitre.

Ans. Goitre is defined as enlargement of thyroid gland.

Sequence of events in development of goitre (Flowchart 20.5):



FLOWCHART 20.5. Sequence of events in development of goitre.

1. **Diffuse nontoxic (simple) goitre:** Diffuse enlargement of the thyroid gland without nodularity.

Types

(a) **Endemic**
- (i) Common in **Alps, Andes and Himalayas** (labelled endemic when more than 10% of the population is affected)
- (ii) Dietary supplements decrease incidence
- (iii) Variation in prevalence of goitre in regions with similar levels of iodine deficiency indicates the existence of other dietary influences, called **goitrogens**, which may influence the prevalence rate.
- (iv) Goitrogens, eg, **vegetables of Brassicaceae and Cruciferae families (cabbage, cauliflower, Brussels sprouts, turnip and cassava)** and excessive calcium in the diet, interfere with thyroid hormone synthesis.

(b) **Sporadic**
- (i) Less common than endemic goitre; females are affected more often than males.
- (ii) Seen at the onset of pubertultsy or in young adults.
- (iii) Associated with hereditary enzyme defects and ingestion of goitrogens; not corrected by dietary supplements. There are four major types of enzyme defects:
  - Iodide transport defect
  - Organification defect **(Pendred syndrome)**
  - Dehalogenase defect
  - Iodotyrosine coupling defect

Morphology

Two morphological stages are identified, namely:
- Stage of hyperplasia
  - Diffuse and symmetric enlargement of the thyroid
  - Follicles are lined by crowded columnar cells with piling up of epithelium and formation of pseudopapillary projections
  - Variable colloid content in the follicles
- Stage of involution
  - Starts if the dietary iodine increases or demand for thyroid hormones decreases
  - Follicular epithelium involutes and becomes flattened

- Flattened follicular epithelium with abundant colloid results in an enlarged, colloid rich gland (**colloid goitre**).

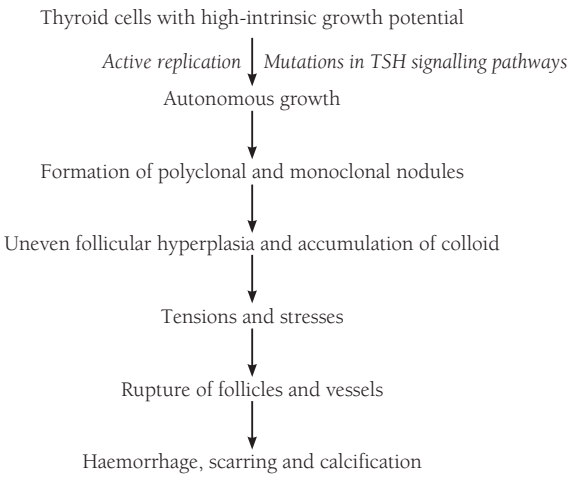
Clinical features

- Patients are usually clinically euthyroid
- Main symptoms are due to mass effects

2. **Multinodular goitre (MNG)**

- Repeated episodes of hyperplasia and involution lead to irregular enlargement of thyroid with formation of nodules.
- MNG may be nontoxic or toxic depending on the secretion of T_3 and T_4.
- Normal thyroid cells are heterogeneous with respect to response to TSH and ability to replicate. Thyroid cells with high-intrinsic growth potential replicate actively.

Steps in the evolution of MNG are given in (Flowchart 20.6):



FLOWCHART 20.6. Evolution of a multinodular goitre.

Gross morphology (Fig 20.1)

- Multinodular, asymmetrically enlarged thyroid
- May exert lateral pressure on midline structures (trachea and oesophagus)
- Growth behind sternum and clavicles labelled '**intrathoracic or plunging goitre**'

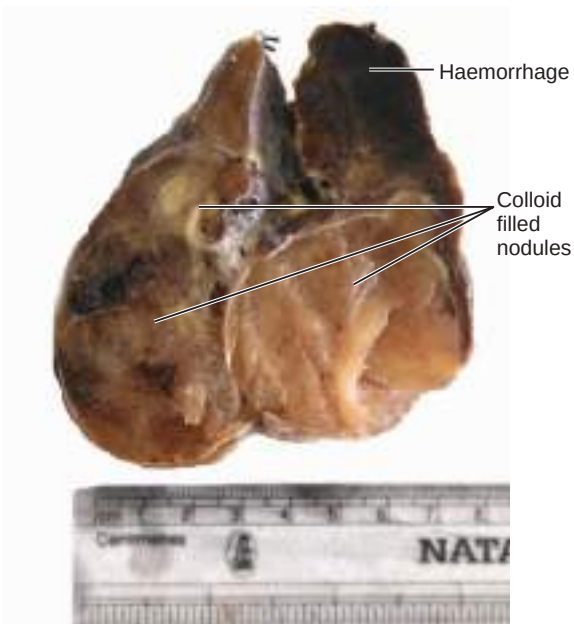


FIGURE 20.1. Gross picture of a MNG showing multiple nodules of variable size.

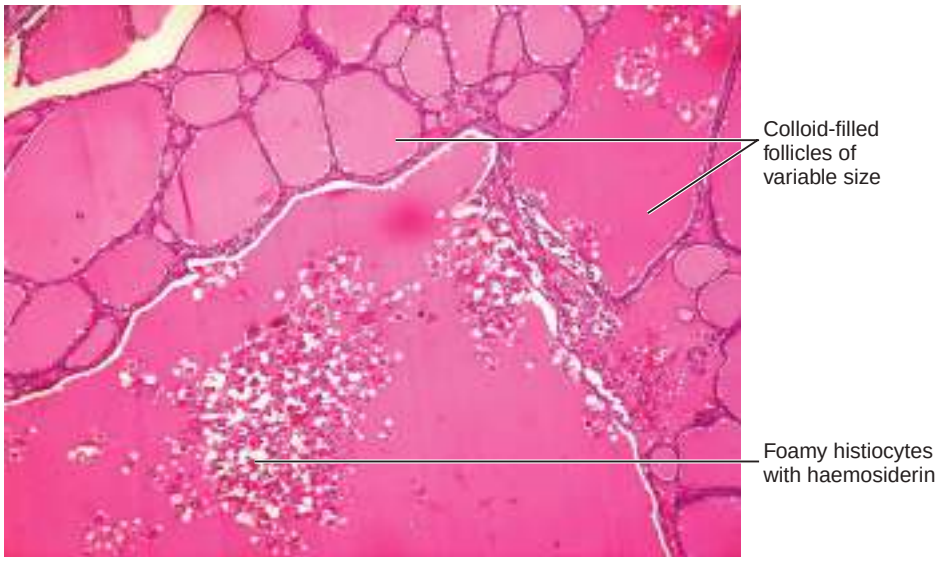


FIGURE 20.2. High power view of a nodule showing follicles of varying size lined by flat to cuboidal epithelium. There is a focus of haemorrhage with aggregates of haemosiderin-laden macrophages (H&E; 100X).

Cut surface:
- Irregular nodules showing a variable amount of brown gelatinous colloid.
- Regressive changes like haemorrhage, calcification, fibrosis and cystic change.

Microscopy (Fig 20.2):
- The nodules consist of colloid-filled follicles of varying size lined by flat to cuboidal epithelium. The colloid-filled follicles may fuse to form large colloid-filled cysts.
- In the background of an enlarged multinodular thyroid, a solitary dominant or hyperplastic nodule may show follicular hyperplasia and hypertrophy (called **nodular adenomatous goitre**).
- Adenomatous goitre can be confused with a follicular adenoma; however, the latter shows a prominent capsule which is lacking in an adenomatous nodule.

Clinical features
- Patients are usually euthyroid and present with an asymptomatic mass in the neck. Some patients may have subclinical hyperthyroidism and decreased TSH levels and others may develop frank hyperthyroidism or toxic multinodular goitre (**Plummer syndrome**).
- Main symptoms due to mass effects (airway obstruction due to compression of trachea; dysphagia due to compression of the oesophagus; venous congestion of the head due to compression of superior vena cava and hoarseness due to recurrent laryngeal nerve compression).

Q. Enumerate the salient features of a solitary nodule of thyroid.

Ans. A solitary thyroid nodule is a palpable discrete swelling within an otherwise normal thyroid gland.

- It is more likely to be malignant than multiple nodules.
- Nodules in younger patients and males are more often malignant than nodules in older patients and females.
- Nodules that do not take up radioactive iodine (cold nodules) are more likely to be malignant.
- Nodules that take up radioactive iodine (hot nodules) are more likely to be benign, eg, nodular (adenomatous) goitre, thyroiditis and simple cysts.

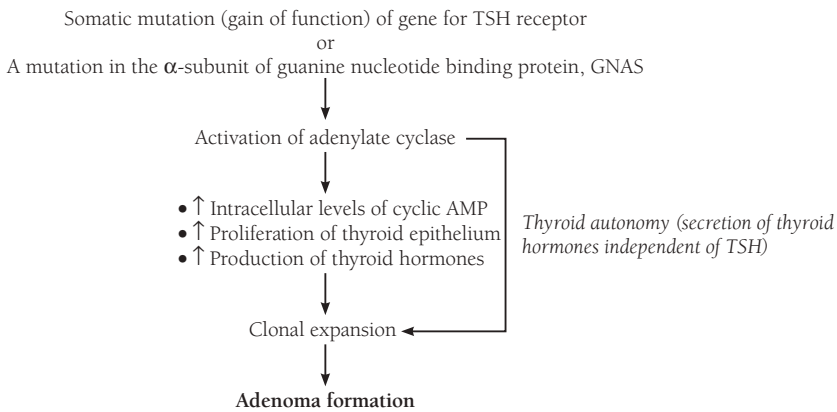
Q. Write briefly on the pathogenesis and clinicomorphological features of adenomas of thyroid.

Ans. Adenomas are discrete, solitary masses derived from the follicular epithelium (thus, also called follicular adenomas). Hormone production in functional adenomas (also called toxic adenomas) is independent of TSH stimulation. This is labelled thyroid autonomy. Majority of the adenomas are nonfunctional (take up less iodine than normal thyroid tissue and appear as cold nodules). Functioning adenomas appear as hot nodules.

Pathogenesis of Nonfunctioning Adenomas
Nonfunctioning adenomas may have any of the following genetic alterations:

1. Mutations in RAS proto-oncogene
2. Phosphatidylinositol-3-kinase subunit abnormalities
3. PAX8-PPARG fusion gene alterations

Pathogenesis of Functioning Adenomas (Flowchart 20.7)



FLOWCHART 20.7. Pathogenesis of functioning adenomas.

Clinical Features
- Unilateral painless masses; variable in size
- Larger masses produce local symptoms, eg, difficulty in breathing and swallowing
- On radionuclide scanning, most adenomas appear as cold nodules
- Definite exclusion of follicular carcinoma is possible only after careful histological examination of capsular integrity.

Gross Morphology
- Solitary, spherical and encapsulated lesions, varying in size from 1 to 10 cm in diameter.
- In fresh specimens, adenomas bulge above the surface and compress the adjacent thyroid.
- Cut surface is grey-white to red-brown with areas of haemorrhage, fibrosis, calcification and cystic change.

Microscopy
Classification of adenomas is based on:
- Presence and size of follicles
- Degree of cellularity
- Amount of colloid

Types
1. Macrofollicular or colloid adenoma
2. Microfollicular or fetal adenoma
3. Embryonal or trabecular adenoma
4. Hürthle cell or oxyphil (oncocytic) adenoma
5. Atypical follicular adenoma (presence of endocrine atypia but absence of capsular invasion)

6. Adenoma with papillae
7. Clear cell and signet-ring adenoma

Q. Differentiate between an adenomatous nodule and a follicular adenoma.

Ans. Differences between an adenomatous nodule and a follicular adenoma are tabulated in Table 20.1.

TABLE 20.1. Differences between an adenomatous nodule and a follicular adenoma

S. No.	Features	Adenomatous nodule	Follicular adenoma
1	Nodules	Multiple	Solitary
2	Encapsulation	Poor	Good
3	Size of follicles within the nodules	Variable	Uniform
4	Morphology of adjacent thyroid	Similar	Architecture is different within and outside the nodule
5	Compression of adjacent gland	Not present	Present

Q. Classify malignant lesions of thyroid. Describe the pathogenesis, clinicopathological features and prognosis of various thyroid malignancies.

Ans. Malignant lesions (carcinoma) of thyroid are mostly seen in adults (papillary carcinoma may be seen in children). Females more commonly affected than males.

Subtypes
- Papillary carcinoma (>85%)
- Follicular carcinoma (5–15%)
- Medullary carcinoma (<5%)
- Anaplastic carcinoma (5%)

Pathogenesis

Contribution from genetic and environmental factors:

1. **Genetic factors**
- Genetic abnormalities in the three follicular epithelium-derived malignancies are observed in two major pathways; namely, mitogen-activated protein (MAP) kinase pathway and phosphatidylinositol 3-kinase (PI3K/AkT) pathway.
- In normal cells, these pathways are transiently activated by binding of soluble growth factor ligands to extracellular domain of receptor tyrosine kinases resulting in autophosphorylation of the cytoplasmic domain of the receptor allowing intracellular signal transduction.
- In thyroid carcinoma, gain of function mutations along these pathways lead to continuous activation, promoting carcinogenesis. Examples include:

(a) Follicular carcinoma
- Abnormalities in PI3K/AKT signalling pathway due to:
  - RAS mutations → NRAS (most common), HRAS, KRAS
  - Mutations in PTEN tumour suppressor.
- Formation of **PAX8-PPAR** (peroxisome proliferator-activated receptor) gamma 1 fusion product due to translocation (2; 3) (q13; p25), which is a nuclear hormone receptor and induces terminal differentiation of cells.

(b) Papillary carcinoma (PTC)

MAP kinase pathway is the major pathway involved in PTC and abnormalities in this pathway can occur by the following mechanisms:
- Rearrangements of the tyrosine kinase receptors (TKRs), RET or NTRK1 (neutrophilic tyrosine kinase receptor 1) due to inversion of chromosome 10 or a reciprocal

translocation between chromosomes 10 and 17 → formation of RET/PTC fusion gene or NTRK1 fusion gene → activation of MAP kinase pathway.
- Mutations in signal transduction genes (RAS mutations and mutations in BRAF oncogene).

(c) Medullary carcinoma
- Sporadic in 80% cases; remainder occur in a setting of MEN IIA or IIB or as familial tumours not associated with MEN syndrome.
- Familial tumours occurring in MEN Type II are associated with germline mutations in RET protooncogene which leads to constitutive activation of tyrosine kinase receptor and cellular proliferation.

2. **Environmental factors**
- Association with ionizing radiation
- Pre-existing thyroid pathology, eg, nodular goitre, adenomas and Hashimoto thyroiditis.

Papillary Thyroid Carcinoma

Clinical features
- Most common thyroid malignancy
- Peak incidence between 20 and 40 years; may be seen at any age
- Presents as a solitary (cold) nodule
- In most cases, primary thyroid nodule is asymptomatic and cervical lymph node metastasis is the first manifestation.
- Primary thyroid nodule may sometimes manifest with hoarseness, dysphagia, cough and dyspnoea.

Predisposing factors
- Previous exposure to ionizing radiation
- Increased incidence of PTC is observed in Gardner syndrome (familial adenomatous polyposis coli) and Cowden disease (familial goitre and skin haematomas)

***Gross morphology*:**
- Solitary or multifocal; often cystic
- May be well circumscribed/encapsulated or ill-defined/infiltrative
- On cut surface, papillary areas are easily identified and appear granular. Areas of fibrosis may be seen

Microscopy (Fig 20.3):
- Branching true papillae with fibrovascular cores covered by multiple layers of cuboidal epithelium (to be differentiated from hyperplastic or pseudopapillae, which do not show true fibrovascular cores).

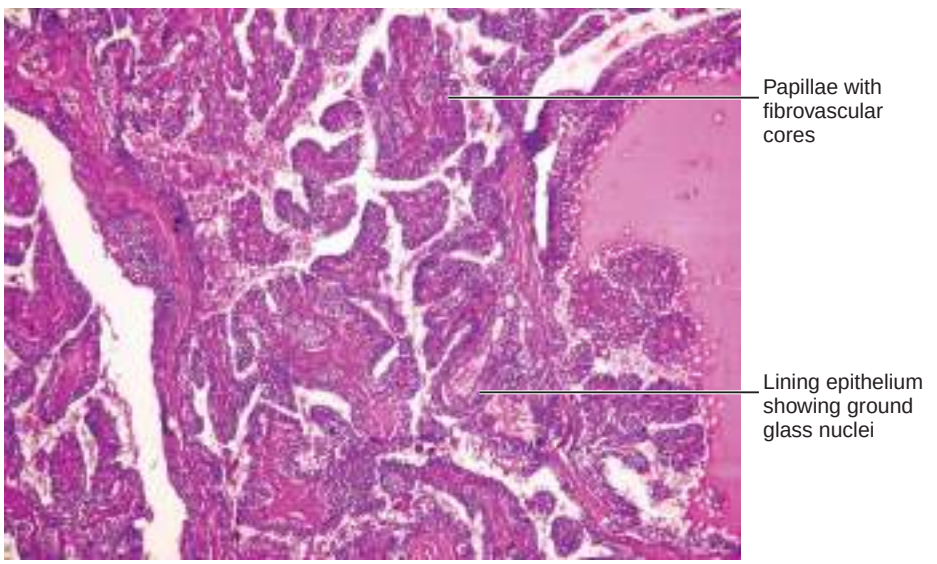


FIGURE 20.3. H&E-stained section from PTC showing branching papillae covered by multiple layers of cuboidal epithelium showing a finely dispersed chromatin, imparting an optically clear or empty appearance to the nuclei **(Orphan Annie or ground glass nuclei; 100X).**

- Cells show finely dispersed chromatin, imparting an optically clear or empty appearance to the nuclei **(Orphan Annie** or **ground glass nuclei).**
- Invaginations of the cytoplasm may in cross-sections give an appearance of eosinophilic **intranuclear inclusions** or **pseudoinclusions** or **intranuclear grooves.**
- Concentrically calcified structures called **psammoma bodies**, usually located within cores of papillae, are often seen.
- **Lymphatic invasion is common**; however, involvement of blood vessels is relatively rare.

Note: Diagnosis of PTC is based on nuclear features irrespective of the presence or absence of papillary architecture.

Variants
- **Encapsulated variant:** Well encapsulated, vascular or lymph node dissemination rare, and excellent prognosis
- **Follicular variant:** Unencapsulated tumours with a follicular architecture, characteristic nuclear features of PTC and psammoma bodies
- **Tall cell variant:** Neoplastic epithelium is tall columnar with intensely eosinophilic cytoplasm. Large tumours, often present with vascular invasion and local and distant metastases. Older individuals have a worse prognosis. Hürthle cell neoplasms are a close differential.
- **Diffuse sclerosing variant:** Younger individuals including children are affected; show diffuse fibrosis, abundant psammoma bodies and squamous morules (metaplasia).
- **Hyalinizing trabecular tumour:** Organoid growth (resembles extra-adrenal paraganglioma), both intra- and extracellular hyalinization are seen.

Prognosis
- Ten-year survival 98%
- Metastasis seen in 10–15% cases

Follicular Carcinoma

Clinical features
- Second most common thyroid carcinoma
- Peak incidence between 40 and 50 years; females more commonly affected than males
- Incidence higher in areas with iodine deficiency; indicating that follicular carcinoma might arise from nodular goitre
- No definite evidence that follicular carcinoma arises from adenomas except for common RAS mutations
- Presents as a slowly enlarging painless cold nodule
- Regional lymph nodes rarely involved; vascular invasion common with spread to bones, lungs and liver

Gross morphology:
- Solitary nodule; may be well circumscribed or infiltrative
- Grey-tan-pink, translucent (due to large colloid-filled follicles)
- Degenerative changes, eg, central fibrosis and foci of calcification are common.

Microscopy:
- Most tumours show a follicular pattern; in some cases, follicular differentiation is less apparent; trabecular pattern, sheets of polygonal to spindle-shaped cells and Hürthle cells are more prominent.
- Anaplasia is variable (generally not marked).
- Blood vessels are preferentially invaded than lymphatics.

Types
1. Minimally invasive
2. Widely invasive (extensive invasion of adjacent thyroid parenchyma or extra-thyroidal tissue)

Prognosis
- Most follicular carcinomas are treated with total thyroidectomy followed by administration of radioactive iodine. Better differentiated lesions are treated with thyroid hormones to suppress endogenous TSH (better-differentiated lesions are stimulated by TSH).

- Widely invasive tumours commonly develop metastasis, and about 50% patients succumb to their disease within 10 years.
- Minimally invasive follicular carcinoma has a 10-year survival greater than 90%.

Medullary Carcinoma

- Neuroendocrine neoplasm derived from the parafollicular or 'C cells'.

Clinical features

- Secretes calcitonin, which has an important role in diagnosis and postoperative follow up of patients.
- In addition, may secrete other polypeptide hormones, eg, somatostatin, serotonin and vasoactive intestinal peptide (VIP).
- Sporadic lesions are common in adults (40–50 years); cases associated with MEN syndrome are seen in younger patients/childhood.
- May present as/due to:
  - A paraneoplastic syndrome, eg, diarrhoea due to excessive VIP or hypocalcaemia due to increased serum calcitonin
  - Mass symptoms

***Gross morphology*:**

- Solitary/multiple lesions seen in both lobes of thyroid
- Bilateral and multicentric in a familial setting, and solitary and unilateral in a sporadic setting
- Firm, pale grey-tan and infiltrative
- Foci of haemorrhage and necrosis may be seen in larger lesions

***Microscopy*:**

- Composed of polygonal to spindle-shaped cells, which may form nests, trabeculae and follicles; rarely small, more anaplastic cells are the predominant cell type.
- Acellular amyloid deposits (derived from altered calcitonin) may be seen in the stroma.
- Multicentric C-cell hyperplasia is often seen in the surrounding thyroid in familial medullary carcinoma thyroid (absent in sporadic medullary carcinoma).
- Electron microscopy shows membrane-bound, electron-dense granules.

***Prognosis*:** Prognosis of familial cancers is worse than sporadic (familial cancers tend to be multiple and are associated with C-cell hyperplasia or micromedullary carcinomas <1 cm).

Anaplastic Carcinoma

It is an undifferentiated tumour derived from thyroid follicular epithelium.

Clinical features

- Presents as a **r**apidly enlarging bulky neck mass, which spread to contiguous structures
- Seen in older patients (mean age of 65 years)
- Fifty percent patients have a previous history of multinodular goitre
- Twenty percent have a previous history of a differentiated carcinoma
- Twenty to thirty percent have a concurrent differentiated thyroid tumour most commonly PTC

$$\text{Differentiated tumours} \xrightarrow[\text{Loss of } P_{53}]{\text{Genetic defects}} \text{Anaplastic carcinoma}$$

Morphology

Highly anaplastic tumour, which may show any of the following histological patterns:

- Giant cell pattern (large pleomorphic giant cells)
- Spindle cell (sarcomatoid) pattern
- Mixed spindle cell and giant cell pattern
- Small cell pattern

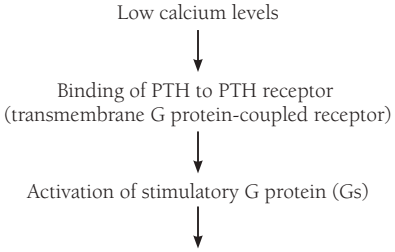
***Prognosis*:** Commonly metastasizes to lungs; is aggressive and fatal.

PARATHYROID GLAND

- Derived from developing pharyngeal pouches that also give rise to thymus
- Four glands (two each at upper and lower poles of thyroid)

- Yellow-brown, ovoid, encapsulated, measuring 35–40 mg
- Composed of:
  - Chief cells (secrete PTH or parathormone)
  - Oxyphil cells (appear at the onset of puberty, but have no known function)

Regulation of parathormone secretion (Flowchart 20.8).
Activity of parathyroid is controlled by levels of free ionized calcium in the bloodstream.



Note: Abnormalities of G protein result in hyper- or hypofunctioning of parathyroid.

FLOWCHART 20.8. Regulation of parathormone secretion.

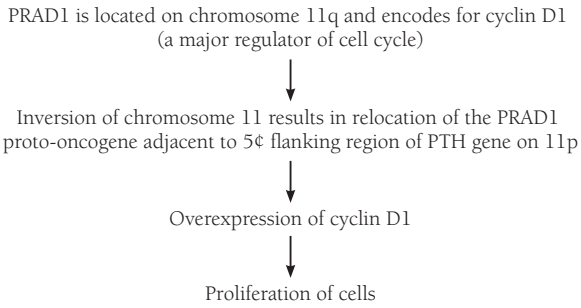
Metabolic functions of PTH
- Activates osteoclasts, mobilizes calcium from bone to blood
- Increases renal tubular reabsorption of calcium and conserves free calcium
- Increases conversion of vitamin D to its active form in the kidneys
- Increases urinary phosphate excretion, lowering the serum phosphate levels
- Enhances gastrointestinal calcium absorption

Q. Classify hyperparathyroidism. Describe the salient clinicopathological features of the various types of hyperparathyroidism.

Ans. Hyperparathyroidism is classified into:

1. **Primary hyperparathyroidism**
   (a) One of the most common endocrine disorders associated with autonomous spontaneous overproduction of PTH and hypercalcaemia, primary hyperparathyroidism is a disease of adults with a female:male ratio 5-3:1.
   (b) Parathyroid lesions causing its hyperfunction include
      (i) Adenomas (75–85% cases); may be familial or sporadic
      (ii) Primary hyperplasia (10–15% cases)
      (iii) Parathyroid carcinoma (5–10% cases)
   (c) In more than 95% cases, disorder is caused by sporadic parathyroid hyperplasia or parathyroid adenoma; less than 5% cases are familial.

Parathyroid adenoma
Pathogenesis
- Genetic syndromes associated with familial primary hyperparathyroidism are
  - MEN-1 (**Werner syndrome**): Tumour suppressor gene on chromosome 11q13 inactivated
  - MEN-2A: Mutations in tyrosine kinase receptor RET on chromosome 10q
  - Familial hypocalciuric hyperkalaemia (FHH): It is an autosomal dominant disorder in which patient show enhanced parathyroid function due to decreased sensitivity to extracellular calcium because of mutations in PTH calcium-sensing receptor gene (CASR) on chromosome 3q.
- Sporadic parathyroid adenomas are mostly monoclonal and associated with two molecular defects:
  - *Parathyroid adenomatosis gene 1 (PRAD1)* (Flowchart 20.9)
  - MEN 1 mutations: Mutations in both copies of MEN 1 gene are seen in up to 30% sporadic adenomas.



FLOWCHART 20.9. PRAD1-associated evolution of a sporadic parathyroid adenoma.

Gross morphology:
- Parathyroid adenomas are always solitary, lie in close proximity to thyroid or in ectopic sites, eg, mediastinum.
- They weigh about 0.5–5.0 g, are well circumscribed, soft tan-reddish brown, with a delicate capsule.
- Gland outside the adenoma may be normal in size or shrunken due to feedback inhibition by increased serum calcium.

***Microscopy*:**
- Predominantly composed of fairly uniform, polygonal chief cells with small centrally placed nuclei. Few nests of oxyphil cells may be seen scattered.
- Pure oxyphil adenomas are rare.
- Mild endocrine atypia may be seen and should not be interpreted as a malignancy.

Primary parathyroid hyperplasia
- Occurs sporadically or as a component of MEN syndrome.
- Classically, all four glands are involved; frequent asymmetry with sparing of one or two glands may be seen.
- Most common pattern is chief cell hyperplasia, which may involve the gland in a diffuse or multinodular pattern.
- Rarely, constituent cells contain **'water clear cells'** (water clear cell hyperplasia). Chief cells appearing clear due to loss of glycogen are called water clear cells.

Parathyroid carcinoma
- May be circumscribed or clearly invasive, grey-white and irregular mass
- Nodular or trabecular arrangement of cells resembling normal parathyroid cells
- Diagnosis of carcinoma is based on invasion of adjacent tissue or metastases.

Morphological changes in other organs due to primary hyperparathyroidism

Skeletal changes
- Prominent osteoclasts (cause erosion of bone matrix)
- Increased osteoblastic activity (induces formation of new bony trabeculae)
- Cortex is grossly thinned; marrow shows an increase in fibrous tissue with foci of haemorrhage and cyst formation **(osteitis fibrosa cystica)**
- Aggregates of osteoclasts, reactive giant cells and haemorrhagic debris, labelled **Brown tumour of hyperparathyroidism**, are typically encountered.

Renal changes

Formation of urinary tract stones (nephrolithiasis)

Clinical features of primary hyperparathyroidism:
- Mostly asymptomatic with deranged biochemical findings:
  - ↑ Serum calcium
  - ↑ PTH levels
  - ↑ Urinary excretion of both calcium and phosphate
- Symptomatic patients may manifest with:
  - Bone pain, fractures, osteoporosis and osteitis fibrosa cystica
  - Nephrolithiasis, pain, obstructive uropathy and chronic renal insufficiency
  - GIT disturbances like nausea, constipation, peptic ulcers, pancreatitis and gall stones
  - CNS alternations, like depression, lethargy and seizures

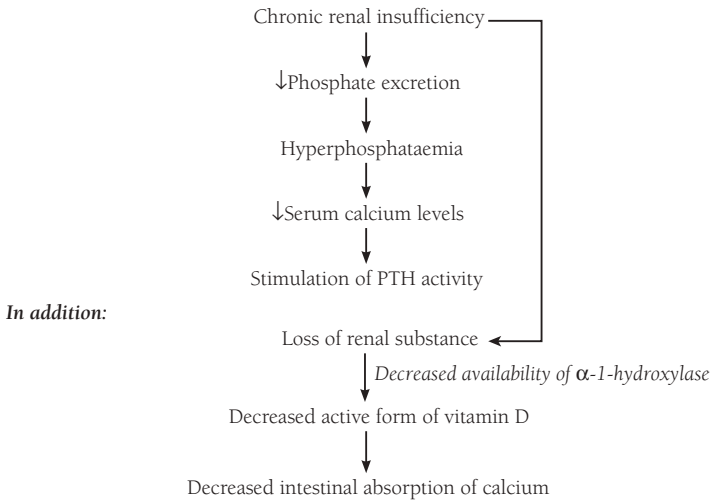
- Neuromuscular abnormalities, like weakness and fatigue
- Cardiac manifestations, like aortic or mitral valve calcification

2. **Secondary hyperparathyroidism**

It may be caused by any condition which is associated with chronic hypocalcaemia.

Causes:

- Renal failure
- Inadequate dietary intake of calcium
- Steatorrhea
- Vitamin D deficiency



FLOWCHART 20.10. Mechanism of development of secondary hyperparathyroidism.

Mechanism is complex, not fully understood (Flowchart 20.10):

Clinical features:

- Manifestations of chronic renal failure
- Bone abnormality **(renal osteodystrophy)** is seen but is less severe than primary hyperparathyroidism.
- Vascular calcification leads to ischaemia **(calciphylaxis).**

3. **Tertiary hyperparathyroidism.**

In a minor population, parathyroid activity may become autonomous and excessive, a process sometimes referred to as tertiary hyperparathyroidism.

Q. Describe the causes and clinicopathological features of the various types of hypoparathyroidism.

Ans. Hypoparathyroidism is far less common than hyperparathyroidism.

Causes

- Congenital absence
- Surgical ablation
- Familial hypoparathyroidism (autoimmune polyendocrine syndrome, Type I):
  - Mutation in the autoimmune regulator (AIRE) gene
  - Associated with mucocutaneous candidiasis and primary adrenal insufficiency
- Idiopathic hypoparathyroidism
  - Autoimmune disease with isolated atrophy of the parathyroid
  - Sixty percent of these patients have antibodies against CASR (calcium-sensing receptors)

Clinical features

Presents mainly with manifestations of hypocalcaemia:

- Tetany, characterized by neuromuscular irritability (circumoral numbness, paraesthesias of the distal extremities, carpopedal spasm, laryngospasm and generalized seizures)
- Mental status changes including emotional instability, anxiety, depression, confusion, hallucinations and frank psychosis
- Intracranial manifestations include calcification of basal ganglia, Parkinson-like movement disorder and increased intracranial pressure with resultant papilloedema
- Ocular disease (calcification of the lens resulting in cataract formation)
- Cardiovascular manifestations including conduction abnormalities
- Dental abnormalities (dental hypoplasia, failure of eruption, defective enamel and root formation and abraded carious teeth)

ADRENAL GLANDS

- Paired endocrine organs
- Weigh about 4 g in adults
- Acute stress leads to lipid depletion, which causes decreased weight of the gland
- Three components:
  - Capsule
  - Cortex: Composed of:
    - Zona glomerulosa
    - Zona fasciculata (broad middle zone comprising more than 75% of the cortex)
    - Zona reticularis
  - Adrenal medulla: Composed of chromaffin cells, which synthesize and secrete catecholamines

'Adrenal cortex' synthesizes three different types of steroids:

1. Glucocorticoids synthesized in zona fasciculata and zona reticularis
2. Mineralocorticoids synthesized in zona glomerulosa
3. Sex steroids synthesized in zona reticularis

Q. Write briefly on the pathogenesis, clinical features and morphology of Cushing syndrome.

Ans. Cushing syndrome is a state of hypercortisolism (increased glucocorticoid levels).

Pathogenesis

Endogenous Cushing syndrome

- Primary hypothalamic–pituitary disease associated with hypersecretion of ACTH, also called **'Cushing disease'** (constitutes 70–80% cases of endogenous Cushing syndrome)
- Hypersecretion of cortisol by an adrenal adenoma, carcinoma or nodular hyperplasia called '**ACTH-independent Cushing syndrome**' (constitutes 10–20% cases of endogenous Cushing syndrome)
- Secretion of ectopic ACTH by a neuroendocrine neoplasm called **paraneoplastic Cushing syndrome** seen in small cell carcinoma of the lung, carcinoid tumours, medullary carcinoma thyroid and islet cell tumours of the pancreas

Exogenous or iatrogenic Cushing syndrome

Due to administration of exogenous corticosteroids.

Clinical Features

- Central obesity (upper trunk and back) with moon faces and buffalo hump
- Weakness and fatigability due to selective atrophy of fast twitch (Type II) myofibrils and decreased muscle mass
- Hirsuitism and menstrual irregularities
- Hypertension
- Glucose intolerance/diabetes (induction of gluconeogenesis and decrease in uptake of glucose by cells with resultant hyperglycaemia, glycosuria and polydipsia)
- Osteoporosis and skin striate (catabolic effect on proteins causing loss of collagen and resorption of bone)
- Neuropsychiatric manifestations, eg, mood swings, depression and frank psychosis

Morphology

Main lesion of Cushing syndrome is found in pituitary and adrenal glands.

- Pituitary glands:
  - Changes regardless of the cause of Cushing syndrome
  - Most common alteration due to high levels of glucocorticoids is labelled **Crooke hyaline change** in which the normal granular basophilic cytoplasm of the ACTH-producing cells in the anterior pituitary is replaced by homogenous lightly basophilic material; the alteration is because of accumulation of **intermediate keratin filaments** in the cytoplasm.
- Adrenals: Morphology varies depending on the cause of hypercortisolism. The adrenals have one of the following abnormalities:
  - Cortical atrophy (exogenous glucocorticoids cause suppression of endogenous ACTH)
  - Diffuse hyperplasia
  - Nodular hyperplasia
  - Adenoma
  - Carcinoma

Q. Write briefly on the pathogenesis, clinical features and morphology of hyperaldosteronism.

Ans. Hyperaldosteronism is a generic term for a group of many closely related syndromes, characterized by **excessive aldosterone secretion.** Excessive aldosterone secretion causes sodium retention and potassium excretion resulting in hypertension and hypokalaemia.

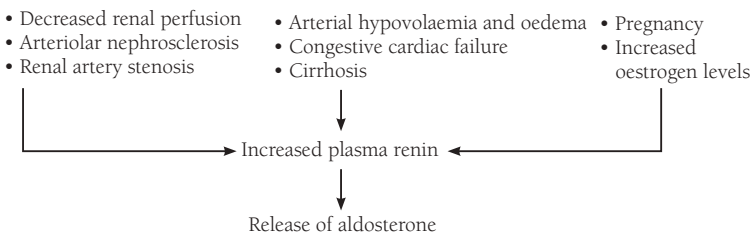
Types

- Primary
- Secondary (due to an extra-adrenal cause)

1. **Primary hyperaldosteronism**
   (a) Autonomous overproduction of aldosterone
   (b) Resultant suppression of renin–angiotensin system and decreased plasma renin activity
   (c) Caused by:
      (i) Adrenocortical neoplasm

Adenoma (80%) or Conn syndrome — Carcinoma (20%)

      (ii) Primary idiopathic adrenocortical hyperplasia, which is characterized by bilateral nodular enlargement.
      (iii) Glucocorticoid-remediable hyperaldosteronism (familial), which is caused by a chimeric gene, resulting from fusion of CYP11B1 (11 β-hydroxylase) and CYP11B2 (aldosterone synthase).
2. **Secondary hyperaldosteronism (**Flowchart 20.11**):** Aldosterone release occurs secondary to activation of renin–angiotensin system.



FLOWCHART 20.11. Pathogenesis of secondary hyperaldosteronism.

Morphology

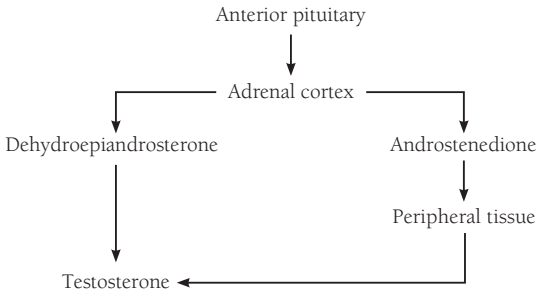
- **Aldosterone-producing adenomas**
  - Solitary, small (less than 2 cm in diameter) and well circumscribed with yellow, cut surface
  - Affect patients in third to fourth decades; females are more commonly involved than males
  - Composed of lipid-laden cortical cells with the presence of eosinophilic, laminated inclusions, known as **spironolactone bodies** found after treatment with the antihypertensive drug **spironolactone.**
- **Bilateral idiopathic hyperplasia**
  - Diffuse or focal hyperplasia of cells resembling those of normal zona glomerulosa
  - Focal hyperplasia is wedge-shaped extending from periphery to centre of the gland

Q. Write briefly on adrenogenital syndromes.

Ans. Adrenogenital syndromes are disorders of sexual differentiation, eg, virilization and feminization, caused by:

1. Primary gonadal disorders
2. Several adrenal disorders

Excess secretion of testosterone (Flowchart 20.12)



FLOWCHART 20.12. Regulation of testosterone secretion.

- Hypersecretion of sex steroids, mainly androgens, occurs as:
  - A pure syndrome
  - Component of Cushing syndrome
- May occur in children or adults
- In children, it is caused by congenital adrenal hyperplasia (total lack of a particular enzyme involved in the biosynthesis of cortical steroids).
- In adults, it is caused by adrenal cortical adenomas and adrenal carcinomas.

Clinical features

- In children: Distortion of external genitalia in girls and precocious puberty in boys.
- In adults: Females show virilization (hirsutism, oligomenorrhoea and deepening of voice) and males show feminization.

Q. Write briefly on the causes, clinical features and morphology of adrenal insufficiency.

Ans. Adrenal insufficiency is classified into:

1. **Primary and secondary insufficiency based on the underlying cause.**
   (a) Causes of primary insufficiency:
      (i) Congenital adrenal hyperplasia
      (ii) Adrenoleukodystrophy
      (iii) Autoimmune adrenal insufficiency

(iv) Infections (AIDS, tuberculosis, fungi and acute haemorrhagic necrosis)
(v) Amyloidosis, sarcoidosis and haemochromatosis
(vi) Metastatic carcinoma

(b) Causes of secondary insufficiency:
(i) Hypothalamic pituitary disease, neoplasms and inflammation (sarcoidosis, tuberculosis, pyogenic and fungal)
(ii) Hypothalamic pituitary suppression (long-term steroid administration and steroid-producing neoplasms)

2. **Acute and chronic insufficiency, based on onset and duration.**
(a) Acute adrenal insufficiency or adrenal crisis:

Causes
- Bilateral adrenalectomy
- Septicaemia, eg, endotoxic shock and meningococcal infection
- Rapid withdrawal of steroids
- Acute stress in chronic deficiency

Clinical features
- Deficiency of mineralocorticoids results in salt deficiency, hyperkalaemia and dehydration
- Deficiency of glucocorticoids results in hypoglycaemia, increased insulin sensitivity and vomiting

(b) 'Chronic adrenal insufficiency' or **Addison disease**: Clinical manifestations do not appear till 90% gland (adrenal cortex) is compromised.

Causes
- Lymphomas
- Amyloidosis
- Sarcoidosis
- Haemochromatosis
- Fungal infections
- Adrenal haemorrhage
- More than 90% cases are due to **autoimmune adrenalitis, tuberculosis and metastatic cancer**

Morphology
- Irregularly shrunken glands are difficult to identify in the suprarenal adipose tissue.
- Cortex contains only scattered residual cortical cells in a collapsed network of connective tissue.
- Variable lymphoid infiltrate may be seen.

Q. Write briefly on Waterhouse–Friderichsen syndrome.

Ans. Uncommon and catastrophic syndrome with the following characteristics:

- May affect any age group but is common in children.
- Usually follows overwhelming bacterial infection due to *Neisseria meningitidis*, *Pseudomonas*, *pneumococci*, *Haemophilus influenzae and Staphylococci*.
- Presents with rapidly progressing hypotension, shock, DIC and widespread purpura due to rapidly progressing adrenocortical insufficiency and massive bilateral adrenal haemorrhage (adrenals converted to sacs of blood).
- Direct bacterial seeding of small vessels in adrenals may lead to DIC.
- Thought to be endotoxin-induced or hypersensitivity-mediated vasculitis.
- Clinical course abrupt; early recognition and institution of appropriate therapy is a must.

Q. Enumerate adrenocortical neoplasms and describe their salient features.

Ans. Adrenocortical neoplasms include

1. **Adrenocortical adenoma**
(a) Indistinguishable from hyperplastic nodules except that hyperplastic nodules tend to be smaller than 2 cm.
(b) Most adenomas are slow growing and nonfunctional; a few larger ones may be functional.

(c) Occasionally, may be a part of MEN-1 syndrome (multiple endocrine neoplasia Type I).
(d) Microscopically, tumour cells are arranged in trabeculae and resemble the cells of zona fasciculata. In a few cases, tumour cells may resemble the cells of zona glomerulosa or reticularis.

2. **Adrenocortical carcinoma**
(a) Rare, can occur at any age including childhood.
(b) More likely to be functional than adenomas.
(c) Large invasive lesions, many exceeding 20 cm in diameter.

Cut surface

- Variegated shows haemorrhage, necrosis and cystic change.
- Invasion of contiguous structures including adrenal vein and inferior vena cava is common. Median survival is two years.

Microscopy

Cells vary from being well differentiated (resembling adenoma cells) to bizarre monstrous giant cells.

Q. Write briefly on the clinicopathological features and laboratory diagnosis of pheochromocytomas.

Ans. Pheochromocytomas are

- Uncommon neoplasms composed of chromaffin cells, which synthesize and secrete catecholamines
- **Important because they cause surgically correctable hypertension**
- Associated with rules of '10'. Ninety percent arise from adrenal medulla, 10% from extra-adrenal tissue (those developing in extra-adrenal paraganglia are called **paragangliomas)**
- Known to be associated with, MEN-IIA, MEN-IIB and von Hippel–Lindau syndromes, as well as von Recklinghausen disease and Sturge–Weber syndrome (Germline mutations in RET, NF1, VHL and succinate dehydrogenase complex subunit or SDHB, SDHC and SDHD genes)
- Ten percent of sporadic adrenal pheochromocytomas are bilateral and 10% are malignant.

Histology

- Polygonal to spindle-shaped chromaffin cells arranged in small nests (Zellballen pattern) or alveoli along with their supporting cells. They are separated by a rich vascular network.
- Finely granular cytoplasm (seen better with silver stain)
- Variable cellular and nuclear pleomorphism; mitotic figures are rare.
- Capsular and vascular invasion may be seen in benign lesions also; a diagnosis of malignancy is exclusively based on the presence of metastasis.

Clinical Course

- Abrupt increase in blood pressure with palpitations, headache, vomiting, sweating, tremors and a sense of apprehension. In two-thirds of patients, hypertension is chronic and sustained.
- Paroxysms are precipitated by stress.
- Cardiac complications include congestive cardiac failure, pulmonary oedema, myocardial infarction, cerebrovascular accidents, myocardial instability and arrhythmias.

Laboratory Diagnosis

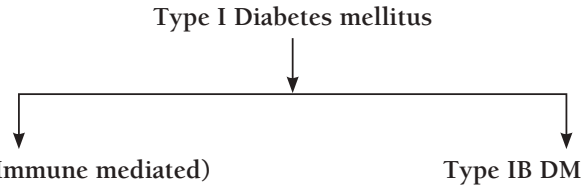
Increased urinary excretion of free catecholamines and their metabolites, eg, vanillylmandelic acid (VMA) and metanephrines, is typically seen.

Q. Define and classify diabetes mellitus (DM). Describe its pathogenesis, clinical features and complications.

Ans. As per WHO, DM is a heterogeneous metabolic disorder, characterized by chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism.

Aetiologic classification of DM **(as per American Diabetes Association):**

1. **Type I DM (10% incidence); earlier called IDDM or juvenile-onset diabetes:** Contrary to its earlier name, this can manifest at any age and is associated with β-cell destruction and absolute insulin deficiency. It is classified into two subtypes.



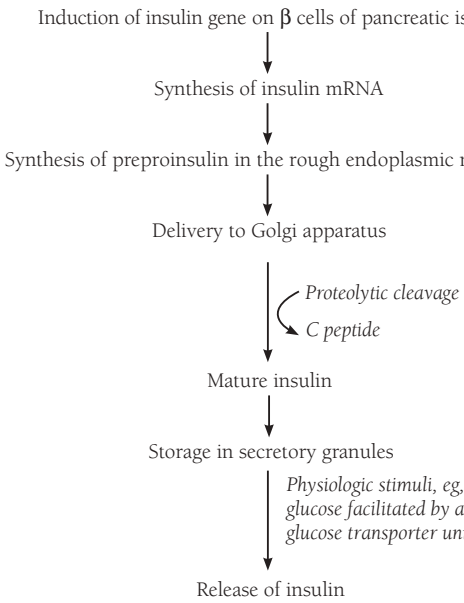
2. **Type II DM** (80% incidence); **earlier called NIDDM** is associated with insulin resistance and relative insulin deficiency.
3. **Genetic defects in β-cell function**
   (a) Genetic defect of β-cell function due to mutations in various enzymes-hepatocyte nuclear factor 4α, MODY1, glucokinase, MODY2; earlier called **maturity onset diabetes of young** or **MODY**.
   (b) Neonatal diabetes (activating mutations in KCNJ11 and ABCC8 encoding Kir6.2 and SUR1, respectively).
   (c) Maternally inherited diabetes and deafness (MIDD) due to mitochondrial DNA mutations
   (d) Defects in proinsulin conversion
   (e) Insulin gene mutations
4. **Genetic defects in insulin action**
   (a) Type A insulin resistance
   (b) Lipoatrophic diabetes
5. **Exocrine pancreatic defects**
   (a) Chronic pancreatitis
   (b) Pancreatectomy/trauma
   (c) Neoplasia
   (d) Cystic fibrosis
   (e) Haemochromatosis
   (f) Fibrocalculous pancreatopathy
6. **Endocrinopathies**
   (a) Acromegaly
   (b) Cushing syndrome
   (c) Hyperthyroidism
   (d) Pheochromocytoma
   (e) Glucagonoma
7. **Infections**
   (a) Cytomegalovirus
   (b) Coxsackie B virus
   (c) Congenital rubella
8. **Drugs**
   (a) Glucocorticoids
   (b) Thyroid hormone
   (c) Interferon α
   (d) β adrenergic agonists
   (e) Thiazides
   (f) Nicotinic acid
   (g) Phenytoin
9. **Genetic syndromes associated with diabetes**
   (a) Down syndrome
   (b) Klinefelter syndrome
   (c) Turner syndrome
   (d) Prader Willi syndrome

10. **Gestational diabetes**

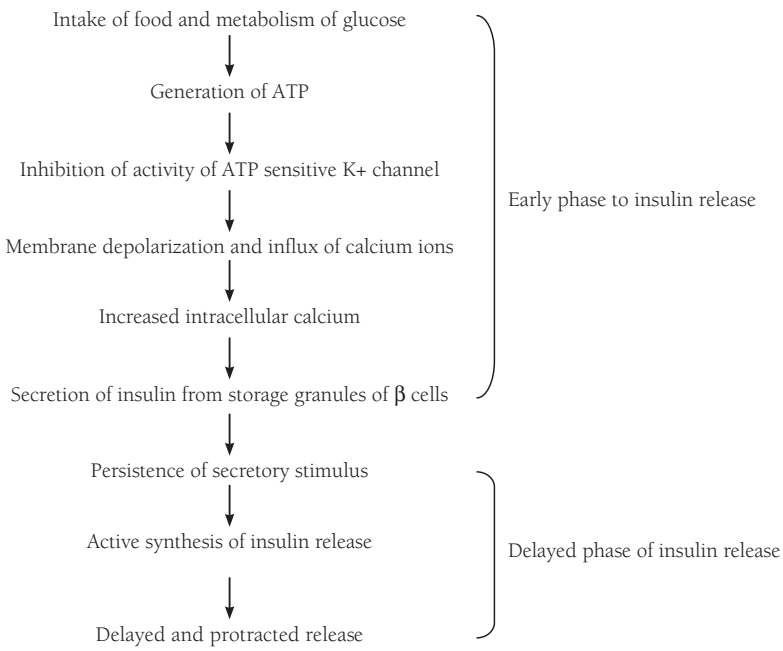
Normal glucose homoeostasis regulation involves three steps:
- Glucose production by liver
- Uptake and utilization by peripheral tissue
- Secretion of insulin and counter-regulatory hormones-like glucagon

Insulin:

1. ***Synthesis and release*** (Flowchart 20.13)



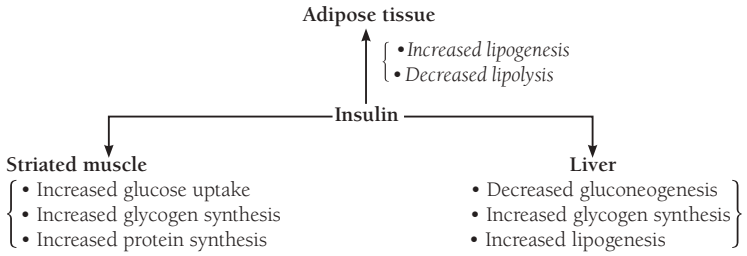
β cells express an ATP sensitive K+ channel on their membrane, which comprises two subunits
a) An ATP-sensitive K+ channel.
b) The sulphonyl urea receptor (binds to this class of hypoglycaemic drugs)



FLOWCHART 20.13. Synthesis and release of insulin.

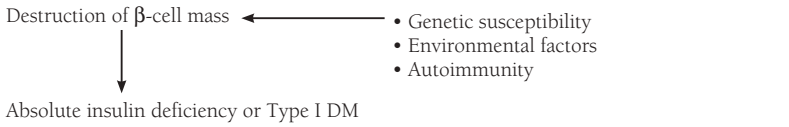
Two factors, namely 'glucose-dependent insulinotropic polypeptide or GIP' secreted by endocrine k cells located in the small bowel and 'glucagon-like peptide-1 or GLP-1Z' secreted by L cells located in distal ileum and colon are released immediately after food intake. They are called **incretins** and their stimulatory effect on secretion of insulin from β cells is labelled '**incretin effect**'. This effect is blunted in Type II diabetes.

2. ***Signalling pathway***
   (a) Insulin receptor is a tetrameric protein composed of two α and two β chains.
   (b) The β subunit cytosolic domain possesses tyrosine kinase activity.
   (c) Insulin binds to the extracellular domain of α subunit to activate the β subunit tyrosine kinase, leading to autophosphorylation of the receptor and phosphorylation of several intracellular substrate proteins, eg, family of insulin receptor substrate proteins (IRS) proteins, which includes IRS1-4 and GAB1.
   (d) The substrate proteins activate multiple downstream signal cascades including PI-3k and MAP kinase pathways, which mediate the several actions of insulin.
   (e) Insulin aids in the docking of glucose transporter unit GLUT-4 to the plasma membrane (GLUT-4 promotes glucose uptake).
3. ***Actions*** (Flowchart 20.14)



FLOWCHART 20.14. Actions of insulin.

Pathogenesis of Type I DM (Flowchart 20.15)



Note: Clinical features of Type I DM manifest after 80% of β-cell mass has been destroyed.

FLOWCHART 20.15. Pathogenesis of Type I DM.

***Factors implicated in destruction of* β-cell mass**

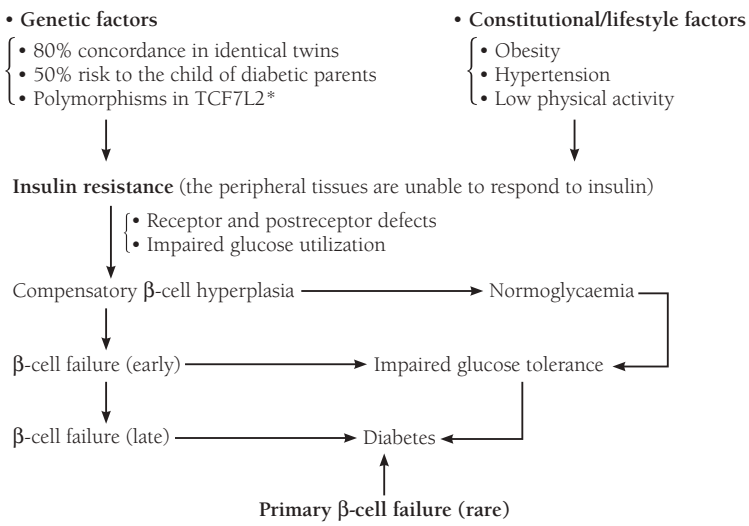
1. Genetic susceptibility.
   (a) Fifty percent concordance in identical twins.
   (b) Susceptibility gene is located on HLA-D region on chromosome 6. Approximately 95% of patients with Type I DM have either human leukocyte antigen (HLA)-DR3 or DR4 haplotype. A concurrent HLA-DQ8 haplotype is considered a specific marker of Type I DM susceptibility.
   (c) Polymorphisms in non-MHC genes like CTL4, PTPN22 and CD25, (which codes for the α chain of IL2 receptor) have been implicated in causation of Type I DM. All three are critical for regulation of T cells.
2. Environmental factors: Type I DM is thought to result from damage to pancreatic beta cells from an infectious or environmental agent. Factors implicated are
   (a) Viruses (eg, mumps, rubella, Coxsackie B4): Three different mechanisms explain the role of viruses in inducing autoimmunity in Type I DM.
      (i) Bystander damage: Viruses induce islet injury leading to release of sequestered antigens and activation of autoreactive T cells.
      (ii) Molecular mimicry: Viruses produce proteins that mimic β-cell antigens and the immune response to viral proteins cross reacts with the self-tissue.
      (iii) Theory of predisposing and precipitating viruses: Viral infection early in life persists (predisposing virus) and a subsequent infection with a related virus

(precipitating virus) that shares antigenic epitopes, leads to an immune response against the infected islet cells.

(b) Toxic chemicals
(c) Exposure to cow's milk in infancy
(d) Cytotoxins
(e) Recent evidence suggests a role for vitamin D in the pathogenesis and prevention of diabetes mellitus.

3. Autoimmune factors: Currently, autoimmunity is considered the major factor in the pathophysiology of Type I DM. Evidence implicating autoimmunity includes
   (a) Circulating islet cell (glutamic acid decarboxylase or GAD and antiinsulin) antibodies
   (b) β cells damage by cytokines (γ IFN, TNF and IL1)
   (c) Prominent insulitis (including cellular necrosis and lymphocytic infiltration)
   (d) Tissue injury caused by macrophages activated by $CD4^+$ T cells
   (e) Direct killing of β cells by $CD8^+$ T cells
   (f) Increased prevalence of Type I DM in patients with other autoimmune diseases, such as Graves disease, Hashimoto thyroiditis and Addison disease.

Pathogenesis of Type II DM (Flowchart 20.16)

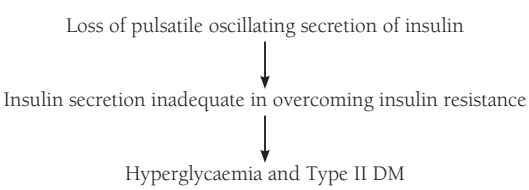


*TCF7L2 encodes a transcription factor in the WNT signalling pathway.

FLOWCHART 20.16. Pathogenesis of Type II DM.

Type II DM can show both quantitative and qualitative defects in β cells;

- ***Quantitative defect in β cells***
  Decreased β-cell mass, islet degeneration and islet amyloid deposition
- ***Qualitative defect in β cells*** (Flowchart 20.17)



FLOWCHART 20.17. Qualitative defect in β cells.

Role of obesity in insulin resistance

- Inverse correlation exists between fasting plasma nonesterified fatty acids (NEFA) and insulin sensitivity. Central fat is more lipolytic. Excess circulating NEFA generated therefore get deposited in the liver and muscle. Intracellular NEFA overwhelms the fatty acid oxidation pathways leading to accumulation of toxic intermediates like diacylglycerol (DAG) and ceramide. These activate the serine/threonine kinases, which cause aberrant serine phosphorylation of insulin receptor and IRS proteins, reducing insulin signalling.
- Adipocytes release **prohyperglycaemic adipocytokines** (including retinol-binding protein 4 or RBP 4 and resistin) as well as **antihyperglycaemic adipocytokines** (leptin and adiponectin). Obesity is associated with a **decrease in adiponectin** contributing to insulin resistance. **Excessive resitin** and **RBP-4** are also associated with insulin resistance.
- Adipocytes also release proinflammatory cytokines which induce insulin resistance by increasing cellular stress.
- PPARγ activation promotes secretion of antihyperglycaemic adipocytokines (leptin and adiponectin).

Clinical Features of DM

Type I DM

- When hyperglycaemia exceeds the renal threshold for reabsorption, glycosuria occurs. Glycosuria induces osmotic diuresis and **polyuria** causing loss of water and electrolytes. Depletion of intracellular water due to water loss and hyperosmolarity (resulting from increased blood glucose levels) triggers osmoreceptors of the thirst centres of brain resulting in intense thirst or **polydipsia.**
- Deficiency of insulin leads to a catabolic state (as insulin is an anabolic steroid). Catabolism of proteins and fat causes a negative energy state, which leads to increased appetite or **polyphagia.**
- The catabolic state dominates over the polyphagia and causes progressive loss of weight and muscle weakness.
- Insulin deficiency coupled with glucagon excess decreases peripheral utilization of glucose and induces abnormally high levels of blood glucose, which result in severe osmotic diuresis and dehydration as well as increased ketone synthesis leading to ketonaemia, ketonuria and ultimately diabetic ketoacidosis (presents with severe nausea, vomiting and respiratory difficulty).

Type II DM

- High portal insulin levels in Type II DM prevent unrestricted hepatic fatty acid oxidation and keep ketone body production in check.
- Osmotic diuresis and resulting dehydration can induce hyperosmolar nonketotic coma especially in case of poor fluid intake.

Complications of DM

- **Macrovascular disease (affects large- and medium-sized muscular arteries):** Accelerated atherosclerosis leading to increased myocardial infarction, stroke and lower extremity gangrene.
- **Microvascular disease (causes capillary dysfunction in target organs):** Most profound effects on retina, kidneys and peripheral nerves resulting in diabetic retinopathy, nephropathy and neuropathy.

Pathogenesis of Complications

1. **Formation of advanced glycation end products (AGE):** Nonenzymatic reaction between intracellular glucose with amino group of intra- and extracellular proteins leads to formation of AGEs. AGEs bind to a specific receptor (RAGE) expressed on inflammatory cells (macrophages and T cells), endothelium and vascular smooth muscle.

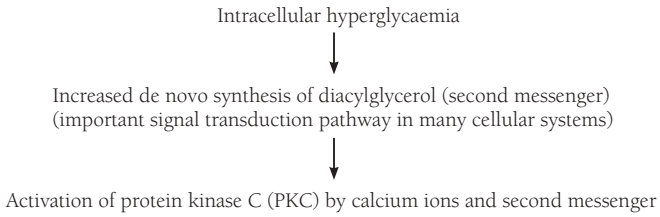
Chemical properties of AGEs

- **AGE crosslink polypeptides of same protein** (crosslinking between collagen Type I molecules in large vessels decreases their elasticity and predisposes the vessel to shear stress and endothelial injury)
- **Trap nonglycated proteins** (trapping of LDL retards its efflux from the vessel wall and enhances the deposition of cholesterol in the intima. In capillaries, albumin binds to the

glycated basement membrane resulting in basement membrane thickening characteristic of diabetic microangiopathy)
- **Shows resistance to proteolytic digestion** (AGE crosslinked proteins are resistant to protein digestion; thus, decreasing protein removal and enhancing protein deposition)

Biologic properties of AGE–RAGE complex
- Leads to generation of reactive oxygen species and NF-κB activation
- Induces monocyte emigration
- Induces cytokine and growth factor secretion
- Increases vascular permeability and procoagulant activity
- Increases ECM production and cellular proliferation

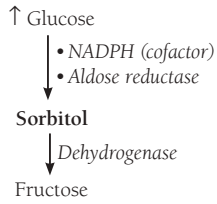
2. **Activation of protein kinase C** (Flowchart 20.18)



FLOWCHART 20.18. Activation of protein kinase C.

Downstream effects of PKC
- Stimulates production of VEGF (induces neovascularization characterizing diabetic retinopathy)
- Increases activity of vasoconstrictor endothelin-1
- Decreases activity of vasodilator nitric oxide synthase (NOS)
- Increases production of profibrogenic molecule TGF-β leading to increased deposition of extracellular matrix and basement membrane material
- Increases production of procoagulant molecule of plasminogen activator inhibitor (PAI-1) leading to decreased fibrinolysis and vascular occlusive episodes
- Enhances formation of proinflammatory cytokines by vascular endothelium

3. **Polyol pathway** (Flowchart 20.19)



FLOWCHART 20.19. Polyol pathway.

- Both sorbitol and fructose are osmotically active and draw water into tissues, leading to permanent damage.
- Complications with osmotic damage include destruction of Schwann cells (causing peripheral neuropathy and cataracts) and damage to pericytes, weakening the vessel wall (causing microaneurysms in diabetic retinopathy).
- NADPH is used as a cofactor by enzyme glutathione reductase for regenerating reduced glutathione (GSH). GSH is an antioxidant and reduction in GSH level increases cellular susceptibility to oxidative stress (NADPH is used as cofactor in polyol pathway).

Morphology of Diabetes and its Late Complications

Pancreas:
- Reduction in number and size of islets (more in Type I than in Type II diabetes)
- Insulitis (leukocytic infiltration of the islets)
- Amyloid replacement of islets in long-standing Type II diabetes

***Vascular system*:**
- Accelerated atherosclerosis (hallmark)
- Gangrene and myocardial infarction
- Hyaline arteriolosclerosis (amorphous hyaline thickening of the wall of the arterioles causing narrowing of the lumen)
- Diabetic microangiopathy (diffuse thickening of the basement membrane, most evident in the capillaries of the skin, skeletal muscle, retina, renal glomeruli, renal tubules, peripheral nerves and placenta)

***Diabetic nephropathy*:**
- *Microalbuminuria*: Earliest manifestation of diabetes is the appearance of low amounts of albumin in urine (>30 mg/day but <300 mg/day).
- Glomerular lesions:
  - Capillary basement membrane thickening
  - Diffuse mesangial sclerosis
  - Nodular glomerulosclerosis **(Kimmelstiel–Wilson lesion; pathognomic of diabetes)**
- Renal vascular lesions: Renal arteriosclerosis and atherosclerosis
- Pyelonephritis including papillary necrosis (necrotizing papillitis)

***Diabetic ocular complications*:**
- Retinopathy
- Cataract
- Glaucoma

***Diabetic neuropathy*:**
Central and peripheral nervous systems are both affected. It alters both motor and sensory functions.

***Defective immunity*:**
- Enhanced susceptibility to infections
- Defects in neutrophilic function

Q. Enumerate the criteria for diagnosis of DM. Enlist the investigations advocated in a patient of DM.

Ans. Diagnostic criteria for diabetes mellitus are described in Table 20.2.

TABLE 20.2. Diagnostic criteria for diabetes mellitus

- HbA1C > 6.5%
- Symptoms of diabetes plus random plasma glucose > 200 mg/dL (symptoms of diabetes plus random whole blood glucose > 175 mg/dL)

  OR
- Fasting plasma glucose > 126 mg/dL; fasting is defined as no calorie intake for at least 8 h (fasting whole blood glucose > 110 mg/dL)

  OR
- Two-hour plasma glucose > 200 mg/dL during an oral 75 g glucose tolerance test (whole blood > 175 mg/dL)

  In the absence of unequivocal hyperglycaemia or presence of acute metabolic decompensation, these criteria should be confirmed by repeat test.

  Impaired fasting glucose (IFG)
- Fasting plasma glucose >110 mg/dL but <125 mg/dL (whole blood glucose >100 mg/dL but <110 mg/dL)

  Impaired glucose tolerance (IGT)
- Plasma glucose between 140 mg/dL and 200 mg/dL, 2 h after oral glucose load (whole blood glucose between 125 mg/dL, 2 h after oral glucose load)

Determination of Blood Glucose

- Glucose concentration is uniform in water phase of plasma and erythrocytes. Since, plasma contains per unit volume 27% more water than erythrocytes, glucose levels are higher in a given volume of plasma than in an identical volume occupied by erythrocytes. For this reason, plasma glucose values are higher than whole blood glucose values.

- In the fasting state, glucose levels in arterial and venous blood are similar. However, postprandially arterial and capillary bloods have glucose levels about 20 mg/dL higher than venous blood. This is because extraction of glucose by tissues in presence of insulin gets elevated in response to nutrient absorption in gastrointestinal tract.
- Quantitative determinations of glucose are based on a variety of chemical and enzymatic methods. The older chemical methods are less specific. Newer enzymatic methods (glucose oxidase, hexokinase) for glucose analysis are highly specific.
- In whole blood, clotted and kept at room temperature, glucose disappears at a rate of approximately 7% per hour owing to ongoing glycolytic activity of leukocytes and red cells. It is thus preferable to collect blood in tubes containing fluoride—a strong inhibitor of glycolysis as well as citrate (acidity) to immediately inhibit glycolysis (grey-stoppered vacutainer tubes).

Oral Glucose Tolerance Test (OGTT)

This test is intended to measure capability and timely response of the insulin-secreting cells to integrated signals provided by GI hormones and rising blood glucose levels.

Patient Preparation

Put the patient for 3 days or more on a normal diet including at least 150 g of carbohydrates per day. In morning after an overnight fast, 75 or 100 g of an aqueous solution of glucose is given.

Steps

1. The patient, who should have been taking an unrestricted carbohydrate diet for at least 3 days or more prior to the test, fasts overnight (at least 8 h).
2. The patient should rest for at least half an hour before starting the test. A sample of blood is drawn to estimate the glucose level.
3. A glucose load of 75 g dissolved in 300 mL of water is given orally.
4. Blood samples are withdrawn at half-hourly intervals for 2 h (½ h, 1 h, 1½ h and 2 h) and glucose levels are estimated.

Interpretation

1. Normal (in nonpregnant adult)
   (a) **Fasting value**: <95 mg/dL
   (b) **Value at 1 h**: <180 mg/dL
   (c) **Value at 2 h**: <155 mg/dL
   (d) **Value at 3 h**: <140 mg/dL
2. Indicative of impaired glucose tolerance (IGT)
   (a) **Fasting value**: 110–126 mg/dL
   (b) At least one of the values at 30, 60 or 90 min >200 mg/dL and value at 120 min between 140 and 200 mg/dL
3. Indicative of diabetes mellitus
   (a) If the fasting glucose determination revealed diabetic values (>126 mg/dL), the OGTT should not be performed
4. If the fasting glucose fell into the IGT range (110–126 mg/dL) and an OGTT is performed, the results are indicative of diabetes mellitus if two or more of venous plasma concentrations are reached or exceeded.
5. The criteria for diagnosis of diabetes during pregnancy (gestational diabetes) are stricter than outlined above for nonpregnant adults. This is because even mild diabetes during pregnancy becomes a significant risk factor for fetal morbidity and mortality. Thus, the OGTT is performed with 100 g of glucose, and it indicates gestational diabetes when two or more of the following values (in mg/dL) are reached or exceeded: fasting 110, 1 h 190, 2 h 165, 3 h 145.

2-h Postprandial Plasma Glucose

This determination has no standardized role for diagnostic purposes. It is, however, often valuable when attempting to optimize patients' treatment. Normally, 120-min values are below 140 mg/dL.

Glycylated Haemoglobin

During the 120-day lifespan of a red cell, haemoglobin A and other forms become glycated due to nonenzymatic, largely irreversible, post-translational attachment of glucose to the α- and β-chains. Degree of glycation is directly proportional to the level of glucose in the blood, and it has been shown that amount of glycated haemoglobin present in blood is a reflection of average blood glucose level over the lifespan of a red cell. Thus, quantitative determination of glycated haemoglobin has become a useful adjunct in assessment of efficacy of long-term therapeutic control of diabetic patients. Glycated haemoglobin can be measured in several ways. The two most common methods are ion exchange and affinity chromatography. When measured by ion exchange, the results are reported as HbA_{1c}
Reference Range: 3.8–6.3%; target for therapy is <7%.

Fructosamine Test

As albumin also contains free amino groups, nonenzymatic reaction with glucose in plasma occurs. Therefore, glycated albumin can serve as a marker to monitor blood glucose. Glycated albumin provides a retrospective measure of average blood glucose concentration over a period of 1–3 weeks. Under alkaline conditions, glycated proteins (ketoamine) reduce nitroblue tetrazolium (NBT) to formazan. In the fructosamine test, absorption of formazan at 530 nm is photometrically measured and compared with appropriate standards to determine the concentration of glycated proteins in plasma, the major part being contributed by albumin.

Determination of Insulin and C-peptide

Insulin is synthesized first as a precursor molecule, proinsulin. The A and B chains in proinsulin are held together by a connecting peptide called C-peptide. Proinsulin is then converted in the β cells to insulin, which is secreted together with C-peptide. Measurements of serum insulin and C-peptide are mostly used to verify classification and for various investigational purposes. Measurements are performed by radioimmunoassay. C-peptide assays are more sensitive than insulin assays because C-peptide levels are not affected by insulin therapy.

Islet Autoantibodies

Markers of cell-mediated autoimmune destruction of islet β cells that can be demonstrated in Type 1 DM are

1. Islet cell antibodies (ICAs)
2. Autoantibodies to insulin (IAAs)
3. Autoantibodies to glutamic acid decarboxylase (GAD65)
4. Autoantibodies to tyrosine phosphatases IA-2α and IA-2β

Population Screening for Type 2 DM

American Diabetes Association (ADA), now recommends this for those at risk of developing DM. The ADA proposes that all asymptomatic people aged 45 years or more, particularly those with BMI ≥25 kg/m^2, should be screened in a healthcare setting. Either FPG, 2-h OGTT or both are appropriate for screening. The FPG is more convenient, more reproducible, less costly and easier to administer than the 2-h OGTT. The FPG is, therefore, the recommended initial screening test. If FPG is <5.6 mmol/L (100 mg/dL) and/or 2-h plasma glucose is <7.8 mmol/L (140 mg/dL), testing should be repeated at 3-year intervals.

Major Risk Factors for Type 2 DM (ADA 2010)

1. Family history of Type 2 DM
2. Obesity
3. Physical inactivity
4. Previously identified impaired fasting glucose or OGTT
5. History of gestational diabetes
6. Hypertension
7. Dyslipidaemia

8. Polycystic ovarian disease or acanthosis nigricans
9. History of vascular disease

Q. Differentiate between Type I and Type II DM.

Ans. General characteristics of Type I and Type II DM are enlisted in Table 20.3.

TABLE 20.3. General characteristics of Type I and Type II DM

Features	Type I DM	Type II DM
Pathogenesis	• Absolute insulin deficiency • HLA-DR3 and DR4 association and contribution from autoimmunity (islet cell antibodies, eg, antiinsulin, anti-GAD); environmental factors also contribute	• Relative insulin deficiency • No HLA association or autoimmune basis • Peripheral tissue resistance secondary to receptor and postreceptor defects (glucose transport abnormal)
Islet cells	• Early insulitis • Marked atrophy and fibrosis • Marked β-cell depletion	• No insulitis • Focal atrophy and amyloid deposits • Mild β-cell depletion
Initial symptoms	• May occur at any age • Manifests with polydipsia, polyuria, polyphagia and weight loss	• Insidious onset in individuals over 40 years • May be symptomatic or asymptomatic
Ketoacidosis	May occur due to lack of insulin	Rare (hyperosmolar nonketotic coma common)
Treatment	Insulin	Diet control and oral hypoglycaemics
Insulin levels	Low or immeasurable	Normal to high
Plasma glucagon	High, suppressible	High, resistant

Q. Write briefly on diabetic emergencies.

Ans. Diabetic coma is a reversible form of a medical emergency seen in diabetes mellitus. Three different types of diabetic coma can occur:

1. Severe diabetic hypoglycaemia
2. Diabetic ketoacidosis (DKA)
3. Hyperosmolar nonketotic coma

Severe Diabetic Hypoglycaemia

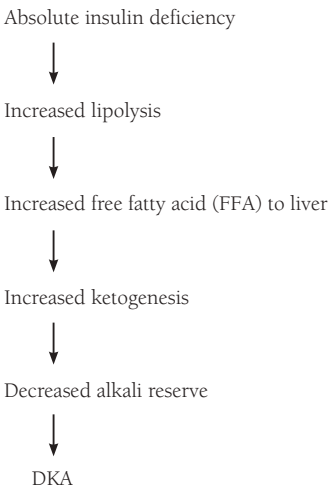
- People with Type 1 diabetes who take insulin in full replacement doses are most vulnerable to episodes of hypoglycaemia.
- Hypoglycaemia is usually mild and easily reversed by eating or drinking carbohydrates, but can be severe enough to produce unconsciousness before it can even be recognized.
- A person suffering from hypoglycaemia is usually pale, and may present with tachycardia, excessive sweating and twitching or convulsions. Unconsciousness due to hypoglycaemia can occur within 20 min to an hour after early symptoms.
- Hypoglycaemic episodes may also result in worsening of diabetic control and rebound hyperglycaemia—a phenomenon called **Somogyi effect**.

Diabetic Ketoacidosis

DKA is a state of absolute or relative insulin deficiency aggravated by hyperglycaemia, dehydration and acidosis. The most common causes are underlying infection, disruption of insulin treatment and new onset of diabetes. Biochemically DKA is defined as:

1. An increase in serum concentration of ketones greater than 5 mEq/L
2. A blood glucose level greater than 250 mg/L
3. A blood (usually arterial) pH less than 7.3
4. Ketonaemia and ketonuria are characteristic, as is a serum bicarbonate level of 18 mEq/L or less is indicative of severe DKA.

Sequence of evolution of DKA (Flowchart 20.20)

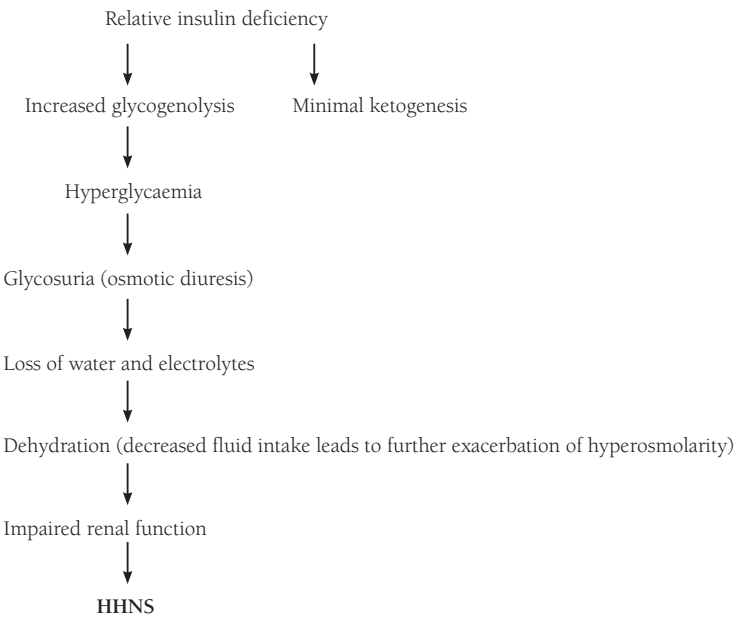


FLOWCHART 20.20. Sequence of evolution of DKA.

Nonketotic Hyperosmolar Coma

- Nonketotic hyperglycaemia is a type of diabetic coma more often associated with a Type 2 DM. The preferred term used by the ADA is hyperosmolar hyperglycaemic nonketotic syndrome (HHNS).
- HHNS is due to severe dehydration resulting from prolonged hyperglycaemia-induced diuresis.
- Osmotic diuresis promotes net loss of electrolytes, including sodium, potassium, calcium, magnesium, chloride and phosphate.
- Intracellular dehydration occurs as hyperglycaemia and water loss leads to increased plasma tonicity, causing a shift of water out of cells. This is associated with movement of potassium from the cell to extracellular compartment.

Sequence of evolution of HHNS (Flowchart 20.21).



FLOWCHART 20.21. Sequence of evolution of HHNS.

Q. Differentiate between DKA and HHNS.

Ans. Differentiating features of DKA and HHNS have been listed in Table 20.4.

TABLE 20.4. Diagnostic criteria and typical total body deficits in DKA and HHNS

Diagnostic criteria	DKA	HHNS
Plasma glucose (mg/dL)	>250	>600
Arterial pH	<7.00	>7.30
Serum bicarbonate (mEq/L)	<10	>15
Urine ketones	Marked increase	Mild increase, if any
Serum ketones	Positive	Small amounts
Effective serum osmolarity	<320	>330
Mental status	Drowsy stupor/coma	Variable
Serum sodium (mEq/L)	Usually low	Normal, increased or low
Serum potassium (mEq/L)	Normal, increased or low	Normal or increased
Serum phosphorus (mEq/L)	Normal or increased	Normal or increased
Serum magnesium (mEq/L)	Normal or increased	Normal or increased
Serum lactate (mmol/L)	2–3	1–2
Blood urea nitrogen (BUN)	Less increase	Greater increase

Q. Define a potential diabetic.

Ans. Potential diabetics are persons with a normal glucose tolerance test, who have an increased risk of developing diabetes for genetic reasons.

Examples
- Children of two diabetic parents
- Sibling of a diabetic
- Nondiabetic member of a pair of monozygotic twins where the other is a diabetic

Q. Define a latent diabetic.

Ans. Latent diabetics are persons in whom the glucose tolerance test is normal but who are known to have given an abnormal result under conditions imposing a burden on the pancreatic cells, eg, during pregnancy, infection, severe stress (physical or mental), during treatment with corticosteroids, thiazide diuretics or other diabetogenic drugs or when overweight.

Q. What is glycosuria?

Ans. Glycosuria occurs when blood glucose level exceeds the renal glucose threshold of 180 mg/dL. Glycosuria can be secondary to hyperglycaemia (diabetes mellitus) or nondiabetic in origin. Nondiabetic glycosuria can be

1. ***Renal glycosuria*:** Glycosuria in the absence of hyperglycaemia due to a lowered renal threshold for glucose.
2. **Alimentary (lag storage) glycosuria:** There is a transient abnormal rise in blood glucose level following a meal, and the concentration exceeds the normal renal threshold. During this time, glucose spills into the urine. This may occur in patients undergoing gastric surgery resulting in rapid gastric emptying, hyperthyroidism or hepatic diseases.

Q. What is gestation diabetes?

Ans. This class of patients is defined as women in whom during pregnancy diabetes or IGT become manifest. After pregnancy, the condition usually reverses to normal; but in some patients diabetes or IGT persists. Untreated gestational diabetes can damage health of fetus or mother. Risks to baby include macrosomia (high birth weight); congenital cardiac, central nervous system and skeletal muscle malformations; respiratory distress syndrome; and red blood cell destruction leading to jaundice.

21

Musculoskeletal System

BONE

Bone is the basic unit of the human skeletal system which is responsible for weight bearing, protection of visceral organs, locomotion and haematopoietic cell production.

Bones can be classified based on their location, size and structure:

Location

Based on location, bones can be classified as follows:

- **Axial skeleton:** Bones of the skull, vertebral column, sternum and ribs
- **Appendicular skeleton:** Bones of the pectoral girdle, pelvis girdle and limbs

Size

Based on size, bones can be classified as follows:

- **Long bones:** They are tubular and hollow with two ends, eg, bones of the limbs.
- **Short bones:** Cuboidal in shape, located only in the foot (tarsal bones) and wrist (carpal bones)

A long bone can further be divided into several regions (Fig. 21.1):

- **Epiphysis:** Region between the growth plate and the expanded end of bone, covered by articular cartilage.
- **Metaphysis:** Region where the growth plate and the diaphysis meet.
- **Diaphysis:** The shaft of long bones located between the two metaphyses.
- **Physis (epiphyseal plate, growth plate):** Separates the epiphysis from the metaphysis and is the zone of endochondral ossification in an actively growing bone.

Structure

1. **Based on texture of cross sections, bone tissue can be classified as follows:**
   (a) **Compact bone (dense bone, cortical bone):** Compact bone is ivory like and dense in texture without any spaces or cavities. It consists mainly of Haversian systems or secondary osteons.
   (b) **Spongy bone (trabecular bone, cancellous bone):** Spongy bone is so named because it is sponge like with numerous cavities. It is located within the medullary cavity and consists of extensively connected bony trabeculae that form a sponge like network. Mature trabecular bone exhibits lamellae and osteocytes between the lamellae. Inactive flattened osteocytes are also present on the bone surface.
2. **Based on matrix arrangement, bone tissue can be classified as follows:**
   (a) **Lamellar bone:** Lamellar bone is mature bone in which the constituent collagen fibres are arranged in lamellae. In contrast to spongy bone, the lamellae are arranged parallel to each other, in compact bone, the lamellae are organized concentrically around a vascular canal (Haversian canal).

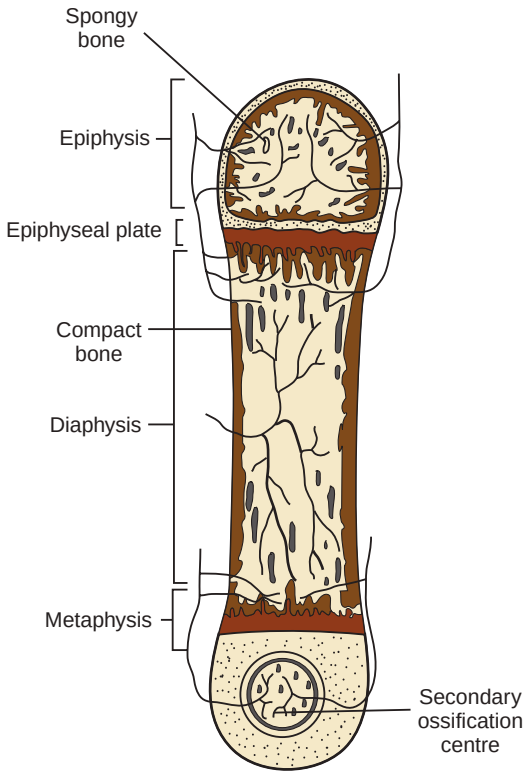


FIGURE 21.1. Parts of a long bone.

(b) **Woven bone:** Woven bone is immature bone, in which collagen fibres are arranged in irregular random arrays and contain smaller amounts of mineral substance and a higher proportion of osteocytes than lamellar bone. Woven bone is temporary and is eventually converted to lamellar bone.

Microscopic Structure of Bone

1. **Bone cells**
   (a) **Osteoblasts:** Line the surface of bone or osteoid and synthesize collagen, proteoglycans and glycoproteins. Osteoblasts also synthesize alkaline phosphatase, an enzyme needed for the mineralization of osteoid. The cell has an eccentrically located nucleus with a prominent nucleolus and a perinuclear halo similar to a plasma cell but lacks the cartwheel-like chromatin pattern that is typical of the latter. An inactive osteoblast has a flattened shape and low alkaline phosphatase activity.
   (b) **Osteocytes:** An osteoblast gives rise to an osteocyte which lies in a lacunar space and is connected to other osteocytes by dendritic processes through tunnels called "canaliculi".
   (c) **Osteoclasts:** Osteoclasts are thought to be derived from the monocyte–macrophage system and are responsible for bone resorption. They are multinucleated cells with fine, finger-like cytoplasmic processes. An increased number of osteoclasts may be seen in diseases with increased bone turnover.
2. **Bone matrix**: Bone matrix consists of organic and inorganic components. The association of organic and inorganic substances gives bone its hardness and resistance. The organic component or osteoid forms 35% and the inorganic or mineral component forms 65% of the bone. Osteoid is composed of collagen fibres with predominately Type I collagen (95%) and amorphous material, including glycosaminoglycans

that are associated with proteins like osteopontin. The inorganic part is constituted by hydroxyapatite which serves as a reservoir for calcium.

Q. Define and classify osteomyelitis.

Ans. Osteomyelitis is defined as infection of the bone (osteo) and marrow (myelo) by bacteria, viruses or fungi.

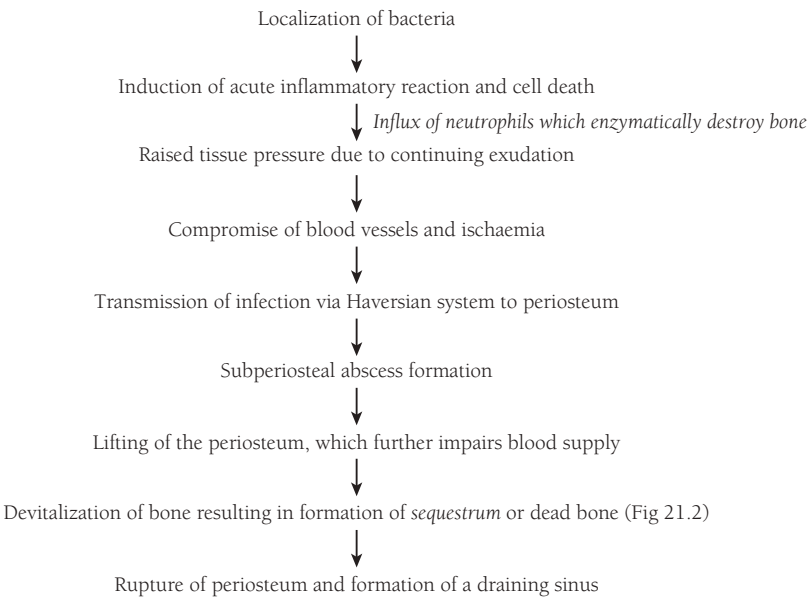
Classification

1. **Pyogenic (bacterial) osteomyelitis:**
   - Most frequently targets children and young adults.
   - Occurs due to haematogenous spread; extension from a contiguous site of infection, eg, cellulitis or direct implantation.
   - Common causative organisms include Staphylococcus aureus (in 80–90% cases) followed by *E. coli, Pseudomonas, Klebsiella, Haemophilus influenzae and Salmonella*. Mixed infections are also seen.
   - The most frequent sites of involvement are the areas of rapid growth (distal femur, proximal tibia, proximal humerus and distal radius).
   - Location of infection is influenced by the vascular circulation. The slowing or sludging of blood flow as the vessels make sharp angles at the metaphyses predisposes the vessels to thrombosis and the bone itself to localized necrosis and bacterial seeding.
   - In the presence of bacterial infection elsewhere, a site of thrombosis acts as a nidus for bacterial growth and development of osteomyelitis.
   - Trauma is an important predisposing factor for osteomyelitis because it aids in venostasis and thrombosis.

***Stages of infection*:**
- **Acute** (develops over days and weeks)
- **Chronic** (develops over weeks to months; may persist for years)

***Pathology*:**
Acute followed by chronic inflammatory cells are seen surrounding fragments of dead bone. Occasionally foreign body giant cell reaction may be seen (Fig 21.3). The dead bone shows empty lacunae without osteocytes.

Sequence of events in osteomyelitis (Flowchart 21.1)



FLOWCHART 21.1. Sequence of events in osteomyelitis

***Complications*:**

1. Draining sinus tract
2. Suppurative arthritis
3. Pathological fracture
4. Secondary ankylosis (fibrosis and fusion of joint)
5. Endocarditis
6. Squamous cell carcinoma with a chronic nonhealing ulcer (Marjolin ulcer)

***Clinical features*:**

Fever, leucocytosis and throbbing pain over the affected region (**differential diagnosis:** is Ewing sarcoma as it may have a similar clinical presentation)

***X-ray*:**

Lytic focus surrounded by sclerosis.

Clinicomorphological variants of osteomyelitis:

Chronic osteomyelitis results from inadequate antibiotic treatment or incomplete surgical debridement. Extensive periosteal reactive bone formation associated with chronic osteomyelitis is called **involucrum**.

Brodie abscess is a small devascularized osteomyelitic focus which becomes encapsulated and surrounded by dense sclerotic reactive bone.

Sclerosing osteomyelitis of Garre typically develops in the **jaw,** and is associated with extensive new bone formation that obscures many of the underlying osseous structures.

2. **Tuberculous osteomyelitis:**

1–3% cases of pulmonary and extrapulmonary tuberculosis present with osseous involvement. Age group affected is adolescents and young adults. Skeletal tuberculosis usually presents as a solitary lesion but may be multifocal in immunodeficient state, eg, AIDS.

***Modes of spread*:**

- Haematogenous (from active visceral disease)
- Direct extension (from a pulmonary focus into the ribs or tracheobronchial nodes into the vertebrae)

FIGURE 21.2 Sequestrum or dead bone showing irregular surface and ragged margins.

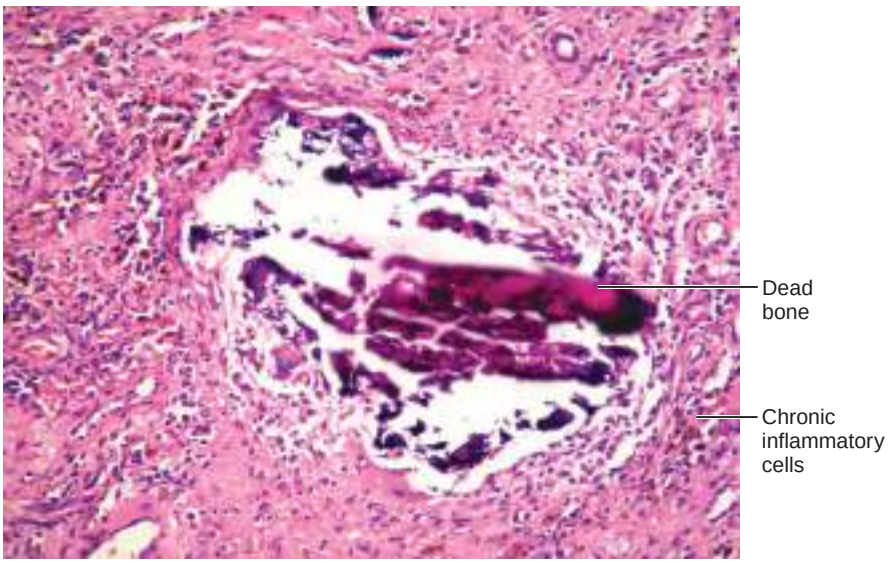


FIGURE 21.3 Photomicrograph of chronic osteomyelitis showing fragments of dead bone surrounded by chronic nonspecific inflammation and foreign body giant cells (H&E; 200×).

***Clinical features*:**
- Commonly affected sites are spine (lumbar and thoracic), knee and hip
- Almost all patient presents with pain, fever and weight loss

***Pathology*:**
Epithelioid cell granulomas with or without necrosis

***Complications*:**
- Psoas abscess
- Fracture
- Neurological deficit and paraplegia due to extension of disease process into dural space with resultant pressure on the cord
- Tuberculous arthritis
- Sinus tract formation
- Ankylosis

3. **Syphilitic osteomyelitis:**
In skeletal syphilis, bone involvement is rare, as disease is readily diagnosed and treated before this stage.

***Clinical features*:**
Skeletal involvement may be
(a) Congenital: Bone involvement starts in fifth month of gestation; manifests with osteochondritis and periostitis as spirochetes tend to localize in areas of active enchondral ossification (osteochondritis) and the periosteum (periostitis).
(b) Acquired: Bone involvement is seen in tertiary stage. Skull and long tubular bones are involved, eg, tibia (massive reactive periosteal bone deposition on medial and anterior surface of tibia is called '**saber shin**').

***Pathology*:**
Necrotic bone is surrounded by chronic inflammatory cells with a predominance of plasma cells

Q. Classify primary bone tumours and describe their salient features.

Ans. Classification of primary bone tumours is given in Table 21.1.

TABLE 21.1. Classification of primary bone tumours

Histological types	Benign	Malignant
Haematopoietic (40%)	–	• Myeloma • Malignant lymphoma
Chondrogenic (22%)	• Osteochondroma • Chondroma • Chondroblastoma • Chondromyxoid fibroma	• Chondrosarcoma
Osteogenic (20%)	• Osteoid osteoma • Osteoblastoma	• Osteosarcoma
Unknown origin (10%)	• Giant cell tumour • Unicameral bone cyst • Aneurysmal bone cyst	• Adamantinoma
Fibrogenic	• Metaphyseal fibrous defect (fibroma) • Nonossifying fibroma • Fibrous histiocytoma • Desmoplastic fibroma	• Fibrosarcoma
Notochordal	Benign notochordal tumour	• Chordoma
Neuroectodermal		• Ewing tumour

Salient Features of Primary Bone Tumours

- They are predominantly seen in the first three decades of life, during the period of greatest skeletal growth.
- Benign tumours are by far more common than the malignant ones. The most common benign tumours are osteochondroma, fibro-osseous lesions and enchondroma.
- Some primary bone tumours are labelled as potentially malignant tumours as they show local aggression but only rarely metastasize, eg, giant cell tumour of bone.
- Among primary malignant neoplasms, multiple myeloma and osteosarcoma have the highest incidence, followed by chondrosarcoma and Ewing sarcoma.
- The commonest sites for primary bone tumours, both benign and malignant, are in distal femur and proximal tibia, which are the bones with the highest growth rate.
- Primary bone tumours have very typical radiographic appearances and a clinico-radiological correlation is a must for correct histopathological diagnosis.

Q. Describe the gross and microscopic features of the common bone-forming tumours.

Ans. Bone-forming tumours are neoplasms in which the constituent neoplastic cells produce bone.

Classification

1. **Benign**
   (a) Osteoma
   (b) Osteoid osteoma
   (c) Osteoblastoma
2. **Malignant**
   Osteogenic sarcoma

Osteoma

Skeletal Distribution

- Flat bones of skull and face
- Paranasal sinuses (frontal and ethmoid)

Clinical Features

- Often asymptomatic; discovered incidentally; occur in middle age; are solitary and slow growing.
- May lead to cosmetic deformity, obstruction of sinus cavity or impingement on brain and eye.

Gross Morphology

- Sessile, round to oval and bosselated
- Project from subperiosteal/endosteal surface of cortex
- Multiple osteomas, may present with intestinal polyposis and soft tissue tumours **(Gardner syndrome)**

Microscopy

Composed of dense and mature lamellar bone.

Osteoid Osteoma

Skeletal Distribution

- Long bones (femur and tibia)
- Usually intracortical; less frequently arise from medullary cavity

Clinical Features

- Common in the age group between 10 and 30 years.
- Presents with **i**ntense pain which increases during night and is relieved by aspirin (pain is attributed to excessive PGE2 produced by proliferating osteoblasts). It may be accompanied by localized swelling and tenderness.

X-ray

Shows a central nidus smaller than 1.5 cm that is surrounded by sclerotic bone. The nidus may be difficult to see on plain X-ray. CT is modality of choice to identify it.

Gross Morphology

Appears as a well-defined, round-to-oval mass of gritty tissue with a size less than 2 cm

Microscopy (*Fig. 21.4*): An osteoid osteoma has two components:

- **Central nidus:** Composed of randomly interconnecting trabeculae of woven bone prominently rimmed by osteoblasts. Stroma surrounding tumour bone consists of loose connective tissue with many dilated-congested capillaries.
- **Envelope:** The nidus is enveloped by sclerotic bone.

Osteoblastoma

Osteoblastoma and osteoid osteoma are histologically very similar, yet these two tumours are very different in their presentation, localization, radiographic appearance, treatment

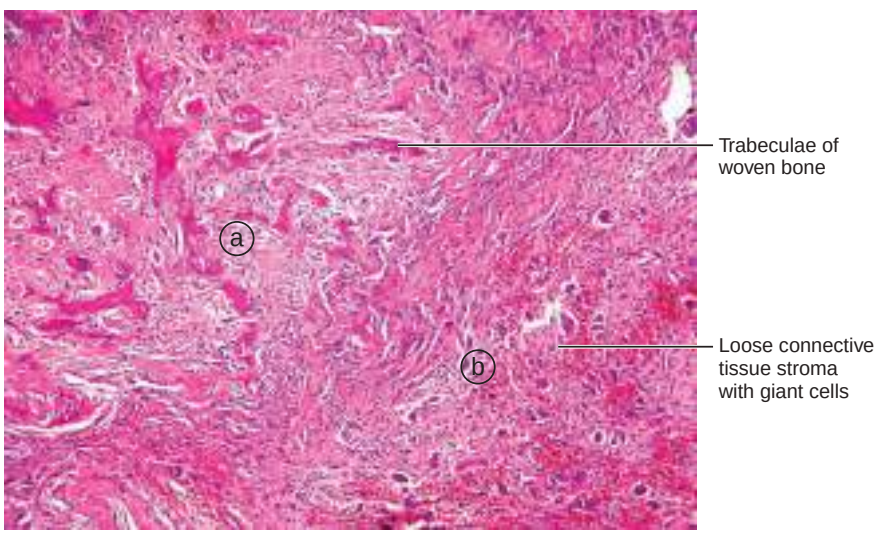


FIGURE 21.4. Section from osteoid osteoma showing a nidus composed of (a) randomly interconnecting trabeculae of woven bone prominently rimmed by osteoblasts and (b) surrounding stroma consisting of loose connective tissue with many dilated-congested capillaries (H&E; 200×)

and potential for recurrence. Osteoblastoma can be differentiated from an osteoid osteoma based on the following features:

- It is larger than 2 cm (also called giant osteoid osteoma).
- Affects older patients.
- Does not cause localized night pain and, when pain occurs, is not relieved by NSAIDs.
- Does not present with as intense a bony reaction as osteoid osteoma.
- Preferentially involves posterior elements of vertebrae, spine, femur and bones of the foot. It is less common in the long bones where it typically involves the metaphysis and may be intracortical or intramedullary in origin.

Osteogenic Sarcoma (OS)

OS is a malignant mesenchymal tumour in which the neoplastic stromal cells directly lay down bone matrix or osteoid without an intervening stage of cartilage formation. It is the most common primary malignant bone tumour after myeloma and lymphoma.

Pathogenesis

1. *Genetic contribution*:
   - Germline mutations in P53 gene (Li–Fraumeni syndrome) increase incidence of OS
   - Mutations in RB gene are seen in 70% of sporadic cases of OS. (Patients with hereditary retinoblastoma have up to 1000 times' greater risk of developing OS.)
   - INK4A is inactivated in some osteosarcomas, INK4A encodes p16 (negative regulator of CDKs) and p14 (which enhances the action of p53).
   - CDK4 and MDM2 are implicated in low-grade osteosarcomas (these are cell cycle regulators which inhibit p53 and RB genes).
2. *Environmental contribution*:

   Radiation, thorotrast and therapeutic irradiation are all implicated. Children treated with alkylating agents have an increased risk of OS.

Classification

1. **Based on affected age and presence of preexisting bone pathology:**
   (a) Primary OS: Arises *de novo* and occurs between 10 and 25 years.
   (b) Secondary OS: Arises secondary to preexisting bone pathology. Occurs in patients more than 40 years and constitutes about 6–10% of all osteosarcomas. Conditions predisposing to secondary OS are
      - Paget disease
      - Exposure to radiation
      - Chemotherapy (alkylating agents)
      - Bone lesions like fibrous dysplasia, osteochondroma, enchondroma, fractures, intramedullary prosthesis and bone infarcts.
2. **Based on skeletal distribution/anatomical site:**
   - Intramedullary
   - Intracortical
   - Surface
3. **Based on morphology:**
   - About 85% of osteosarcomas are of the 'conventional intramedullary' type, and the other 15% consist of several other subtypes, including telangiectatic, low-grade intramedullary and small cell, as well as, the surface subtypes parosteal, periosteal and high-grade surface osteosarcoma.
   - Conventional intramedullary OS is mostly metaphyseal in origin (involves long bones like lower end of femur, upper end of tibia and upper end of humerus, in that order). It can be further classified into the following subtypes depending on the predominant constituent element:
      - Osteoblastic (shows a large amount of osteoid and bony trabeculae)
      - Chondroblastic (malignant cartilage forms nearly 90% of the tumour)
      - Fibroblastic (composed of a large spindle cell/fibroblastic component)

Clinical Features

- Presents as a painful, progressively enlarging mass with a large soft-tissue component, sometimes associated with a pathological fracture.
- OS has a bimodal age distribution

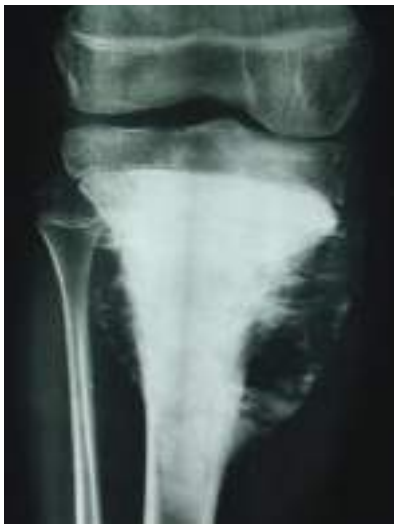


FIGURE 21.5. Plain radiograph showing the typical sunray appearance of osteogenic sarcoma (periosteal reaction perpendicular to cortical surface).

- May show markedly raised levels of serum alkaline phosphatase.

X-ray (Fig. 21.5):

- Conventional OS usually presents as a metaphyseal, large, permeative, destructive, mixed sclerotic-lytic lesion.
- Tumour breaks through the cortex, results in reactive periosteal bone formation and lifts the periosteum. The triangular shadow between cortex and raised periosteum is radiographically called *Codman's triangle*.
- Typically the periosteal reaction is laid down perpendicular to the surface of the bone (*sunray appearance*).

Gross Morphology

- Bulky, gritty and grey-white tumour, often containing areas of haemorrhage and necrosis.
- Destruction of cortex and soft tissue extension are common.
- Penetration of epiphyseal plate/entry into joint is however relatively infrequent.

Microscopy (Fig. 21.6):

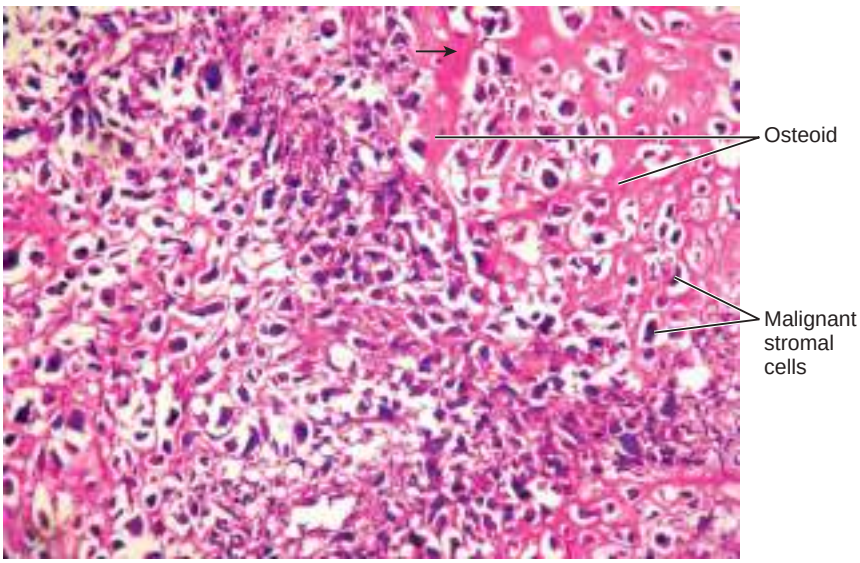


FIGURE 21.6. Sarcomatous stroma composed of large atypical spindle-shaped cells showing direct formation of tumour osteoid, seen as eosinophilic, glassy, homogenous material (H&E; 400×).

- The stroma is frankly sarcomatous, composed of large atypical spindle-shaped cells with bizarre tumour giant cells and frequent mitoses.
- There is direct formation of tumour osteoid by neoplastic cells. Osteoid is arranged in a thin anastomosing lace-like pattern and appears eosinophilic, glassy and homogenous on H&E sections.
- Chondroblastic and fibroblastic elements may also be present besides osteoid.
- Spontaneous necrosis and vascular invasion are frequent.

Surface osteosarcomas

- **Juxtacortical (parosteal)**
  - Slow growing.
  - Classically, located on the posterior aspect of lower femur.
  - Large lobulated mass encircling the bone.
  - Low-grade tumor with a good prognosis.
- **Periosteal osteosarcoma**
  - Grows on the surface of long bones.
  - Occurs on periosteal surface between cortex and periosteum.
  - Prominent cartilaginous component.
  - High-grade osteosarcoma; poor prognosis.
- **Osteosarcoma of jaw**
  - Affects older age.
  - Involves mandible and alveolar ridges of maxilla.
  - Prominent chondroblastic component.
  - Good prognosis.
- **Osteosarcoma in Paget's disease**
  - Multicentric.
  - Pelvis, humerus, and femur bones are involved in that order of frequency.
  - Poor prognosis.

Prognosis

- Lungs, other bones, pleura and heart are common sites of metastases. Regional lymph nodes are however rarely involved.
- Long-term survival rate with chemotherapy and limb salvage therapy is 60–70%.

Q. Describe the gross and microscopic features of the common cartilage-forming tumours.

Ans. Cartilaginous neoplasms of bone are characterized by formation of hyaline or myxoid cartilage.

Classification

Benign:

- Osteochondroma
- Chondroma
- Chondroblastoma
- Chondromyxoid fibroma

Malignant:
Chondrosarcoma

Osteochondroma (Exostosis)

- The most frequent benign bone tumour, osteochondroma presents as a bony outgrowth capped by hyaline cartilage which is attached to the underlying bone.
- Multiple osteochondromas occur in the setting of **multiple hereditary exostoses**, an autosomal dominant condition, which is associated with inactivation of EXT genes (EXT1 and 2). Solitary osteochondromas are thought to arise from displacement of lateral portion of the growth plate.

Skeletal Distribution

Metaphysis of lower femur, upper tibia and upper humerus.

Clinical Features

- Solitary osteochondromas are diagnosed in later life as compared to multiple osteochondromas which usually manifest in childhood itself.
- Osteochondromas are mostly asymptomatic but may present with pain and deformity. They sometimes interfere with the functioning of regional tendons and blood vessels.

X-Ray (Fig. 21.7)

Seen as metaphyseal lesions which grow in a direction opposite to the adjacent joint.

Gross Morphology

May be sessile or pedunculated, mushroom shaped, with an average size of 4–10 cm.

Microscopy (Fig. 21.8)

- The outermost layer is a fibrous membrane, continuous with the periosteum of the adjacent bone.
- Under the fibrous membrane is cartilage cap (which is formed by mature hyaline cartilage).
- Cross-section through the lesion demonstrates mature trabecular and cortical bone.
- The cortex of stalk appears to merge with cortex of host bone.

Complications

- Bursitis (development of bursa around head of a longstanding osteochondroma)
- Formation of osteocartilaginous loose bodies
- Development of secondary chondrosarcoma (incidence of development of secondary chondrosarcoma in solitary osteochondroma is 1–2% and is as high as 10% in multiple lesions)

Chondroma

- Chondroma is the most common intraosseous cartilaginous tumour. Based on location it is classified as intramedullary (also known as enchondroma) and subperiosteal (juxtacortical) chondroma.
- It may be solitary or multiple. Multiple enchondromas can manifest as **Ollier disease** (a rare, nonhereditary disorder characterized by multifocal proliferation of dysplastic

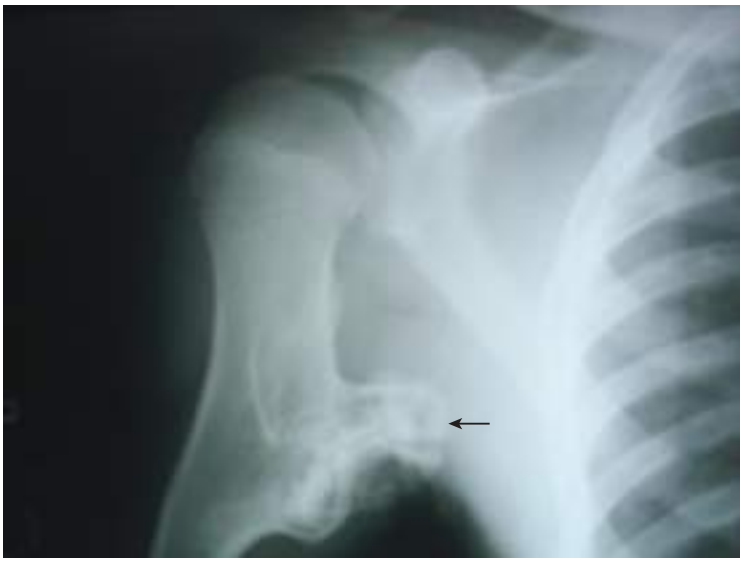


FIGURE 21.7. X-ray showing a lobulated cartilaginous exostosis arising from upper humerus (arrow).

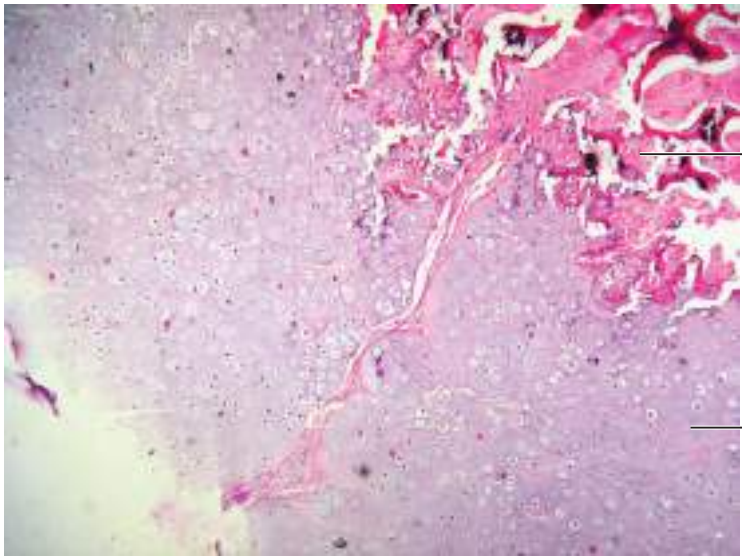


FIGURE 21.8. Osteochondroma composed of outermost fibrous layer, followed by a cartilage cap, underlying which mature trabecular and cortical bone can be seen (H&E; 100×).

cartilage, also known as enchondromatosis) or as **Maffucci syndrome** (multiple enchondromas and soft tissue haemangiomas).

- Chondromas are associated with heterozygous mutations in the IDH1 and 2 genes.
- The risk of malignant transformation, usually to chondrosarcoma, is very high (20–30%) in multiple enchondromas.
- They mainly occur in bones that develop from enchondral ossification (thought to develop from rests of growth plate cartilage that proliferate and enlarge).
- Most lesions are asymptomatic (detected incidentally); may occasionally manifest with pain or cause pathological fracture.

X-Ray

- Plain radiograph shows an intramedullary zone of stippled and ring-shaped calcifications.
- Enchondroma characteristically involves the acral skeleton (small bones of the hands and feet) and the long bones (such as femur, humerus, tibia, fibula, radius and ulna).
- In the long bones, the tumour is found in metaphyses and proximal/distal diaphyses.

Gross Morphology

They are usually smaller than 3 cm, grey-blue and translucent.

Microscopy (Fig. 21.9)

- Sections show well-circumscribed nodules of hyaline cartilage containing benign appearing chondrocytes.
- Cartilage in periphery of nodules undergoes enchondral ossification and the centre frequently calcifies and dies.

Chondroblastoma

Clinical Features

- Rare tumour seen in children and adolescents with open growth plates (usually males less than 20 years).
- It is intramedullary in location and involves the epiphyseal ends of femur, humerus, tibia, and small bones of hands and feet.
- Presents with pain, restricted mobility and joint effusion (effusion occurs due to proximity to the joint).

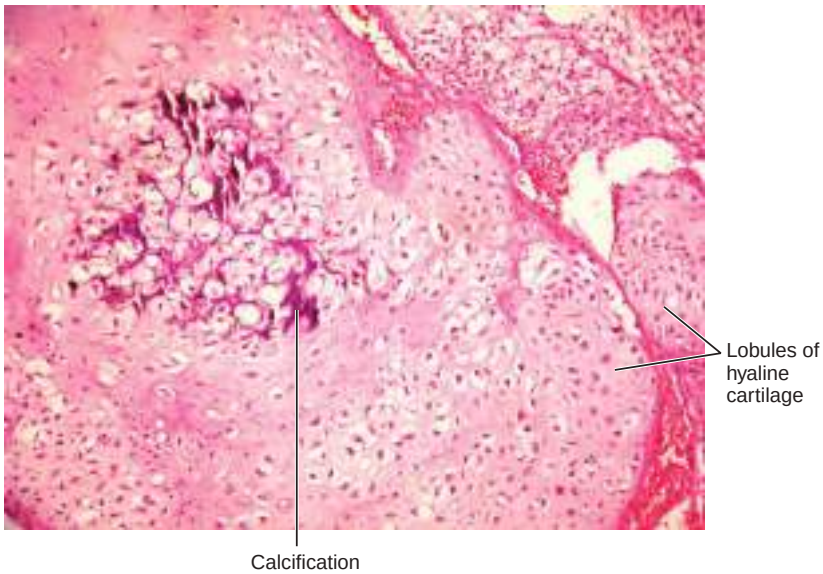


FIGURE 21.9. Section from an enchondroma showing well-circumscribed nodules of hyaline cartilage with cytologically benign chondrocytes. The centre of the nodule shows calcification (H&E; 100×).

X-Ray

Shows a well-defined lytic lesion surrounded by sclerosis. Spotty calcification is common. Cysts are present about 20% of the time and both MRI and CT can define fluid levels.

Gross Morphology

On gross examination, chondroblastoma has a lobulated, round form and is made up of friable, soft, greyish-pink tissue that may be gritty.

Microscopy (Fig. 21.10)

- Extremely cellular tumour composed of closely packed tumour cells.
- The basic tumour cell is an embryonic chondroblast which is a polyhedral cell with sharply defined cell membrane and lobulated nuclei showing longitudinal grooves (**coffee-bean appearance**), without sufficient maturity to produce intercellular chondroid.

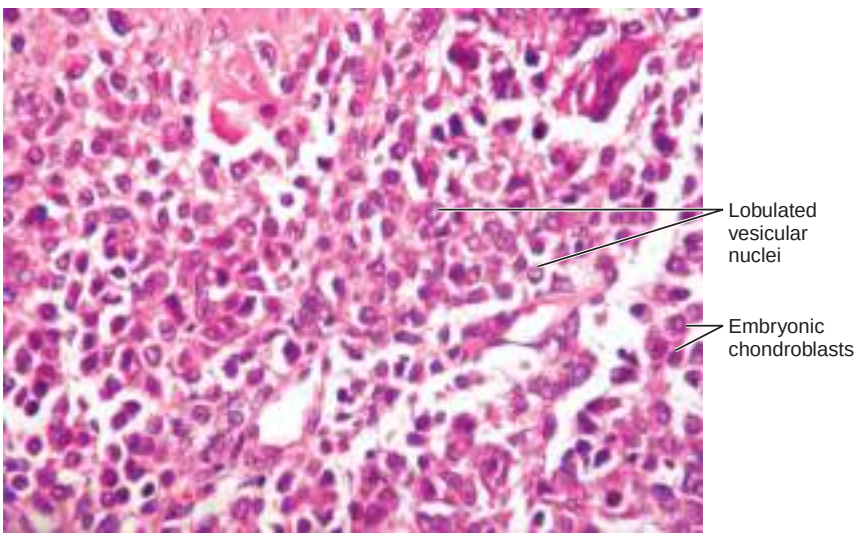


FIGURE 21.10. Section from chondroblastoma showing closely packed clusters of embryonic chondroblasts (polyhedral cells with a sharply defined cell membrane and lobulated nuclei showing longitudinal grooves) (H&E; 200×).

- Mitoses and necrosis are frequent; scattered osteoclastic giant cells may also be seen.
- Scant amount of lace-like hyaline matrix may be laid down, which calcifies to produce a characteristic **chicken-wire calcification.**

Chondromyxoid Fibroma (CMF)

Clinical Features

Affects young adults and presents with localized dull aching pain and swelling in the affected region.

X-Ray

Large, lobulated, sharply defined, eccentric, lytic, metaphyseal lesion surrounded by a rim of sclerosis.

Gross Morphology

Average size is 3–8 cm; cut surface appears solid, glistening and tan-grey.

Microscopy (Fig. 21.11)

- Prominent features of CMF are the zonal architecture and lobular pattern. Hypocellular lobules of poorly formed hyaline cartilage and myxoid tissue are separated by fibrous septae.
- The chondrocytes in myxoid areas are plump-to-spindled in shape and have indistinct cell borders.
- Varying degree of cytological atypia is common along with small foci of calcification.

Chondrosarcoma

It is a malignant mesenchymal tumour that produces cartilaginous matrix. There are several subtypes of chondrosarcoma, which vary in terms of location, appearance, treatment and prognosis.

Classification

1. **Based on pre-existing pathology:**
   (a) Primary chondrosarcoma: Relatively uncommon; arises centrally in the bone, and is found in children

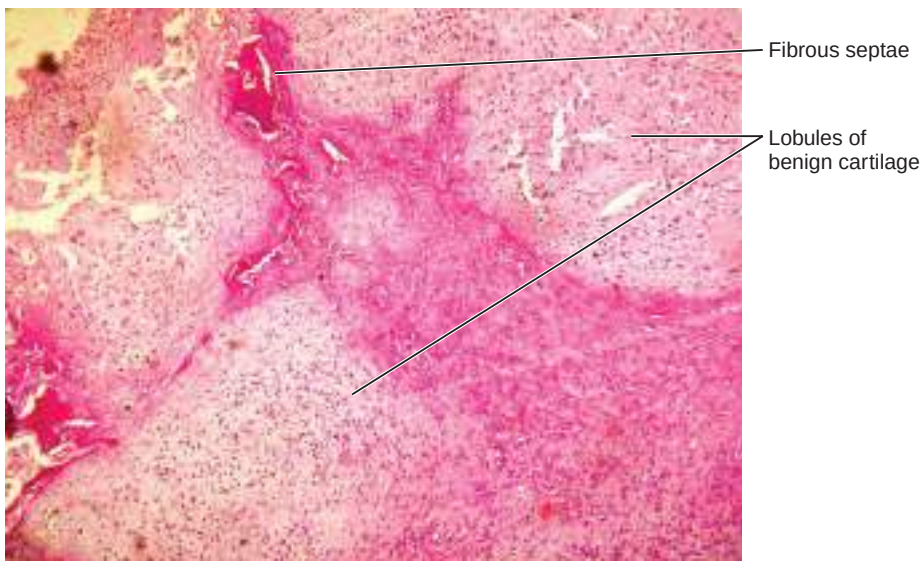


FIGURE 21.11. Hypocellular lobules of poorly formed hyaline cartilage and myxoid tissue separated by fibrous septae; the chondrocytes in the myxoid areas appear plump to spindle with indistinct cell borders (H&E; 100×).

(b) Secondary chondrosarcoma: Arises from benign cartilage defects such as osteochondroma or enchondroma

2. **Based on topography:**
   (a) Conventional intramedullary: Arises from the medullary cavity of long bones, pelvis, costochondral junction of ribs and shoulders and presents as a lytic lesion with blotchy calcification.
   (b) Juxtacortical (peripheral): Arises in the shaft of a long bone.
3. **Based on morphology:**
   (a) Conventional (which is further subtyped as hyaline or myxoid)
   (b) Clear cell
   (c) Dedifferentiated
   (d) Mesenchymal

Gross Morphology

Grey-white, lobulated, bulky, translucent masses with a gelatinous consistency. Erosion/destruction of cortex is frequently seen. Calcification and ossification are not uncommon.

Microscopy (Fig. 21.12)

- Histologically, chondrosarcoma is composed of invasive lobules of anaplastic cartilage and is differentiated from benign cartilaginous tumours based on the following features:
  - Presence of two or more cells per lacuna, binucleate cells, enlarged, plump and hyperchromatic nuclei, nuclear pleomorphism and abundant mitoses.
  - Enchondral ossification is seen (unlike osteosarcoma in which the osteogenesis is directly from malignant stromal cells).
- Chondrosarcoma is classified into Grades I, II and III, based on cellularity, pleomorphism, mitoses and necrosis.

Q. Describe the gross and microscopic features of giant cell tumour of bone.

Ans. Also known as osteoclastoma, GCT is the most common tumour of epiphyses in skeletally mature individuals with closed growth plates. It often shows metaphyseal extension. Common sites include lower end of femur, upper end of tibia and lower end of radius.

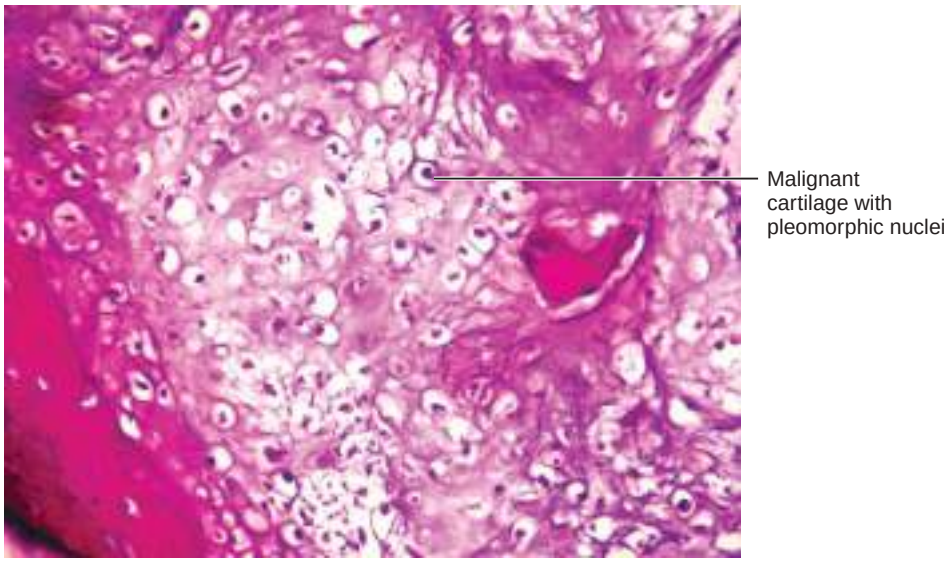


FIGURE 21.12. Section from a chondrosarcoma showing cartilaginous lobules composed of atypical chondrocytes (H&E; 200×).

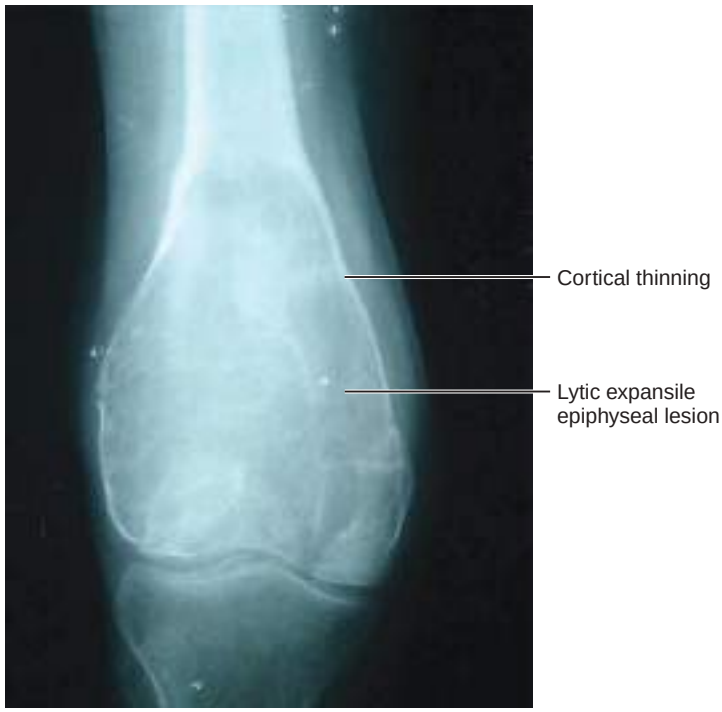


FIGURE 21.13. Radiograph showing a lytic, expansile, epiphyseal lesion in the femur without any sclerosis or periosteal reaction. The cortex shows thinning and destruction. Associated soft tissue mass is a common finding.

X-Ray (Fig. 21.13)

Radiographs show a lytic, expansile, lesion which usually does not show any peripheral sclerosis or periosteal reaction. There is thinning and destruction of cortex with frequent extension into intermuscular septae and joint space.

Gross Morphology (Fig. 21.14)

The tumour is variable sized, solid, tan brown, trabeculated with presence of haemorrhage and necrosis.

FIGURE 21.14. Tan-to-light brown epiphyseal tumour showing abundant haemorrhage and necrosis.

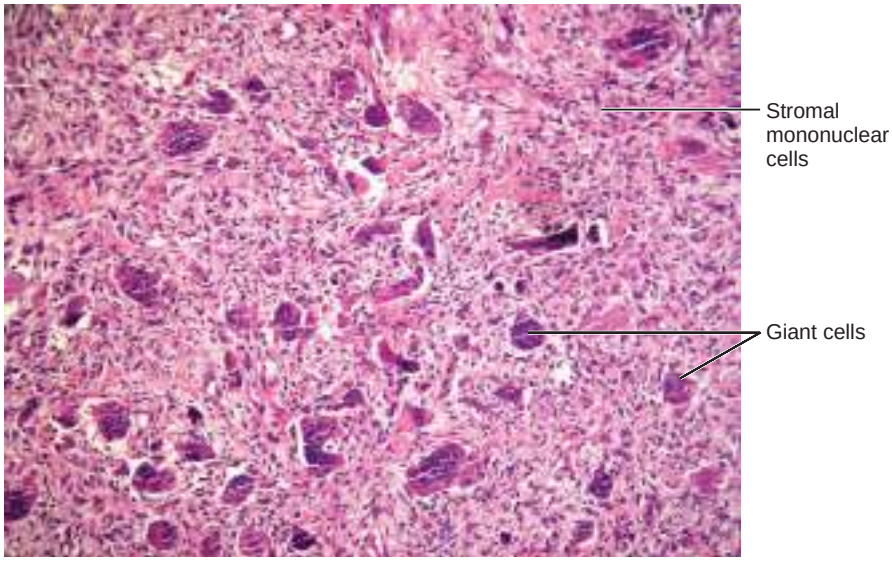


FIGURE 21.15. GCT of bone composed of uniform oval mononuclear cells that grow in a syncytial pattern (stromal element) and giant cells with 20–30 nuclei arranged towards the centre (H&E; 200×).

Microscopy (Fig. 21.15)

- GCT has two histopathological components:
  - *Stromal cells*: Uniform oval mononuclear cells with indistinct cell membrane, which are arranged in a syncytial pattern. They are the basic neoplastic element of the tumour and their number correlates with its clinical evolution.
  - *Giant cells*: Large cells with 20–30 (up to 100) nuclei arranged towards the centre (believed to form via RANK/RANKL signalling pathway).
- Focal deposition of osteoid or bone may occasionally be seen, especially in cases presenting with pathological fracture.
- All GCTs should be regarded as *potentially malignant* (approximately 4% give rise to distant metastasis).

Q. Enumerate the other giant cell containing lesions of bone. How is GCT of bone differentiated from other giant cell containing lesions?

Ans. Other giant cells containing lesions:

1. Metaphyseal fibrous defect
2. Nonossifying fibroma
3. Chondromyxoid fibroma
4. Chondroblastoma
5. Eosinophilic granuloma
6. Solitary bone cyst
7. Osteitis fibrosa cystica
8. Aneurysmal bone cyst
9. Osteoid osteoma
10. Osteoblastoma

Features differentiating GCT of bone from other giant cell-containing lesions are as follows:

- Giant cells in other giant cell-containing lesions have fewer nuclei as compared to GCT bone.

- There is a uniform distribution of giant cells in GCT bone, unlike other giant cell lesions wherein giant cells are focally aggregated.

Q. Describe the clinicopathological features of tumours of neuroectodermal origin.

Ans. Tumours of neuroectodermal origin include the Ewing sarcoma family of tumours (EFST) which further includes Ewing sarcoma (ES) and primitive neuroectodermal tumour (PNET). PNET generally demonstrates a greater neuroectodermal differentiation as compared to ES.

Clinical Features

- ES affects children and young adults (peak incidence between 5 and 20 years; rare after 30 years). PNET is seen in relatively older individuals.
- Both present as a lytic lesion in long bones; most common skeletal sites include femur, tibia, humerus, pelvis and ribs **(A skin tumour of the chest).**
- In the long bones these arise from the medullary canal and are located in the diaphysis or metaphysis.
- Pain, tenderness, swelling accompanied by fever, leucocytosis and elevated ESR are the main presenting features (clinical presentation of EFST mimics chronic osteomyelitis). Rarely, ES may present as a soft-tissue neoplasm without involvement of underlying bone (extraskeletal Ewing sarcoma).

X-Ray

Reactive periosteal bone is laid in layers parallel to cortex **('onion-skin' appearance).**

Microscopy (Fig. 21.16)

- Biopsy shows a highly cellular, infiltrative neoplasm consisting of sheets of tightly packed, round cells with very scant cytoplasm separated into irregular nests/lobules by fibrovascular septae **('round blue cell tumour').**
- The tumour cells appear to be of two distinct types: the larger round cells with a high nuclear/ cytoplasmic ratio, fine chromatin pattern and occasional small, inconspicuous nucleoli and the smaller and darker cells with eosinophilic cytoplasm and hyperchromatic, 'shrunken' nuclei. The latter are actually degenerated cells, a finding typical of Ewing sarcoma.
- The cells have ill-defined cytoplasmic borders with small amounts of vacuolated-to-clear cytoplasm attributed to the presence of cytoplasmic glycogen that gives a granular positivity with PAS stain.
- Necrosis is prominent but tumour giant cells are rare. At places, tumour cells form pseudorosettes **(Homer–Wright rosettes).**

Cytogenetics

Ninety-five percent cases show reciprocal translocation 11:22 (q24:q12). This translocation is common to ES and PNET.

Prognosis

- Haematogenous metastasis to lungs, liver, bones and brain leads to an early spread.
- Disease stage at diagnosis (including tumour volume) is the main prognostic factor for patients with ES/PNET; use of combined chemotherapy and radiotherapy improves clinical outcome.

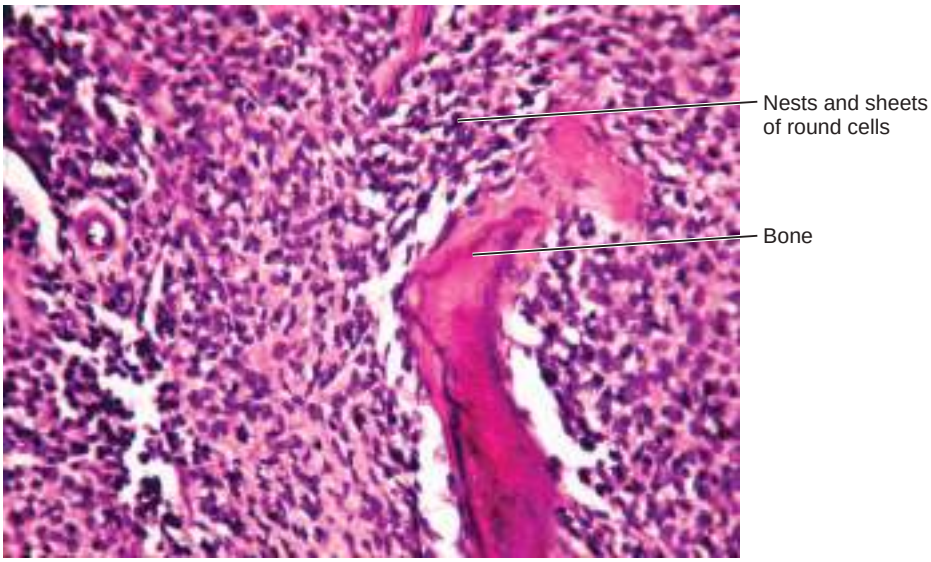


FIGURE 21.16. Section from Ewing sarcoma showing a cellular, infiltrative neoplasm consisting of sheets of round cells with scant cytoplasm arranged in irregular nests/lobules separated by fibrovascular septae (H&E; 400×).

Q. What are the different pathways of spread of tumours to bone?

Ans. Pathways of spread of tumours to bone:

- Direct
- Lymphatic or haematogenous
- Intraspinal seeding

Q. Enumerate the common tumours which metastasize to bone.

Ans. Metastatic cancers are the most frequent malignant tumours found in bone. They are by far more common than primary bone tumours and are characterized by the following features:

- Eighty percent metastases to bone comes from breast, lungs, prostate and kidney. Wilms' tumour, neuroblastoma and rhabdomyosarcoma are the main sources of bony metastases in children.
- Metastasis is usually multifocal and has a predilection for the haematopoietic marrow sites in the axial skeleton (vertebrae, pelvis, ribs and cranium) and proximal long bones. Metastases to long bones distal to the elbows and knees and the small bones of the hands and feet are rare. Occasionally, metastases may appear as solitary lesions (particularly true for lung, kidney and thyroid cancer).
- Carcinoma of prostate, breast and carcinoid tumour gives rise to pure osteoblastic metastases.
- Pure lytic metastases is seen in carcinoma of kidney, lungs, GIT and malignant melanoma.

Q. Enumerate the various cystic lesions of bone. Describe their clinical and pathological features.

Ans. Cystic lesions of bone include

1. **Solitary (simple, unicameral) bone cyst or SBC**
   - Benign lesion occurring in **children** and **adolescents**.
   - Most frequently located in the **metaphysis** of humerus and femur.

- The cyst expands the bone, causing thinning of the overlying cortex.
- Pathogenesis is unknown.
- SBC may remain asymptomatic or present with pain and pathological fracture.

Gross pathology

Generally unilocular with smooth inner lining; filled with yellow or amber coloured fluid.

Microscopy

- Cyst wall consists of thin collagenous tissue having scattered osteoclastic giant cells and newly formed reactive bony trabeculae.
- Fracture may alter the appearance with secondary haemorrhage, haemosiderin deposits and macrophages in the cyst wall.

2. **Aneurysmal bone cyst (ABC)**
   - ABC is an expanding osteolytic lesion filled with blood (aneurysm = dilatation).
   - Common in young patients under **30 years** of age.
   - Most frequently involved is **metaphysis of long bones** or **the vertebral column**.

X-Ray

Characteristic ballooned-out, expansile lesion located underneath the periosteum

Pathogenesis

Not clear; probably arises from persistent alteration in the local haemodynamics

Clinical features

Enlarges over a period of years to produce pain, tenderness and sometimes pathological fracture

Gross pathology

Seen as a large haemorrhagic mass covered over by thinned out reactive bone

Microscopy (Fig. 21.17):

- The cyst consists of blood-filled aneurysmal spaces of variable size, some of which are endothelium-lined.
- The spaces are separated by connective tissue septae, which may contain osteoid tissue and numerous osteoclast-like multinucleate giant cells.
- Histological differentials include GCT and telangiectatic osteosarcoma.

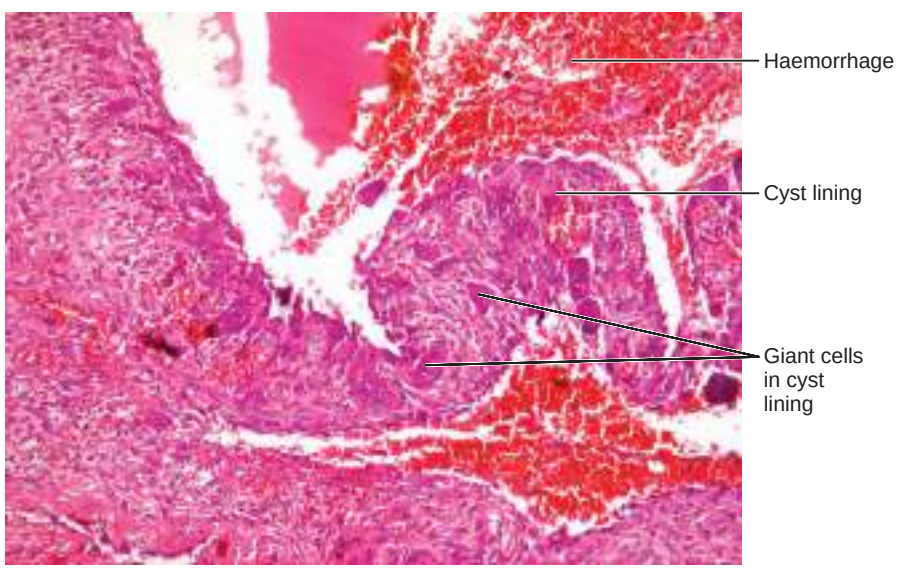


FIGURE 21.17. Photomicrograph of ABC showing blood-filled cystic spaces separated by connective tissue septae which contain osteoid and numerous osteoclast-like multinucleate giant cells (H&E; 100×).

Q. Enumerate the commonly encountered metabolic and endocrine diseases of bone.

Ans. Common metabolic and endocrine bone diseases include

- **Osteoporosis:** Quantitative reduction in otherwise normal bone.
- **Osteomalacia** and **rickets:** Qualitative abnormality due to impaired bone mineralization because of deficiency of vitamin D in adults and children.
- **Scurvy:** Defect in collagen formation caused by the deficiency of vitamin C.
- **Hyperparathyroidism:** Condition in which increased parathyroid hormone (PTH) leads to osteitis fibrosa cystica (OFC).
- **Renal osteodystrophy:** Condition associated with chronic renal failure which results in osteitis fibrosa cystica, osteomalacia and focal osteosclerosis.

Q. Enlist the salient clinicopathological features of osteoporosis.

Ans. ***Clinicopathological features of osteoporosis (osteopenia)*:**

- Common clinical syndrome affecting multiple bones
- Characterized by quantitative reduction of bone tissue mass resulting in a fragile skeleton associated with increased risk of fractures and consequent pain and deformity
- Common in elderly and postmenopausal women
- May be asymptomatic or may manifest with chronic backache; more extensive involvement is associated with fractures, particularly of distal radius, femoral neck and vertebral bodies

Predisposing Factors

- **Genetic factors** (60–80%, variation in bone density genetically determined; associated genes are RANKL, OPG and Receptor Activator of Nuclear Factor κ B (RANK), which are the key regulators of osteoclasts)
- **Sex** (more common in females)
- **Ageing** (decreased replicative and biosynthetic activity of osteoprogenitor cells and osteoblasts with ageing results in senile osteoporosis)
- **Reduced physical activity** (decreases replicative and biosynthetic activity of osteoprogenitor cells and osteoblasts)
- **Starvation** (decreased nutritional intake causes deficiencies)
- **Intake of systemic steroids, anticonvulsants and heparin** (interfere with calcium metabolism)
- **Deficiency of sex hormones** (oestrogen in females and androgen in males), **deficiency of vitamin D** and **hyperparathyroidism.**

Radiology

- Radiological evidence becomes apparent only after more than 30% of bone mass is lost.
- Levels of serum calcium, inorganic phosphorus and alkaline phosphatase are usually within normal limits.

Pathology

- Osteoporotic trabeculae are thinned out with loss of their interconnections.
- Cortex thinned out by subperiosteal and endosteal resorption.
- Haversian system widened; sometimes so much that the cortex mimics cancellous bone.

Q. Enlist the salient clinicopathological features of hyperparathyroidism.

Ans. Hyperparathyroidism may be:

- **Primary:** Due to autonomous hyperplasia and a neoplastic growth (usually adenoma)
- **Secondary:** Caused by a prolonged state of hypocalcaemia

Manifestations (Flowchart 21.2)

Severe hyperparathyroidism of primary or secondary (chronic renal failure) type

↓

Oversecretion of parathyroid hormone

↓

Increased osteoclastic resorption of bone

↓

- Susceptibility to fractures, skeletal deformities and joint pains
- Dysfunction as a result of deranged weight bearing
- Osteitis fibrosa cystica

Note: The bony changes may disappear after cure of primary hyperparathyroidism (removal of functioning adenoma).

FLOWCHART 21.2. Clinicopathological manifestations of hyperparathyroidism

Biochemical Abnormalities

Excessive circulating levels of PTH, hypercalcaemia, hypophosphataemia and hypercalciuria

X-Ray

- Cortical bone affected more severely than cancellous bone, focal areas of erosion of cortical bone (subperiosteal resorption) frequently seen along radial surface of phalanges of index and middle fingers.
- Loss of lamina dura at the roots of teeth is another diagnostic feature.

Pathology

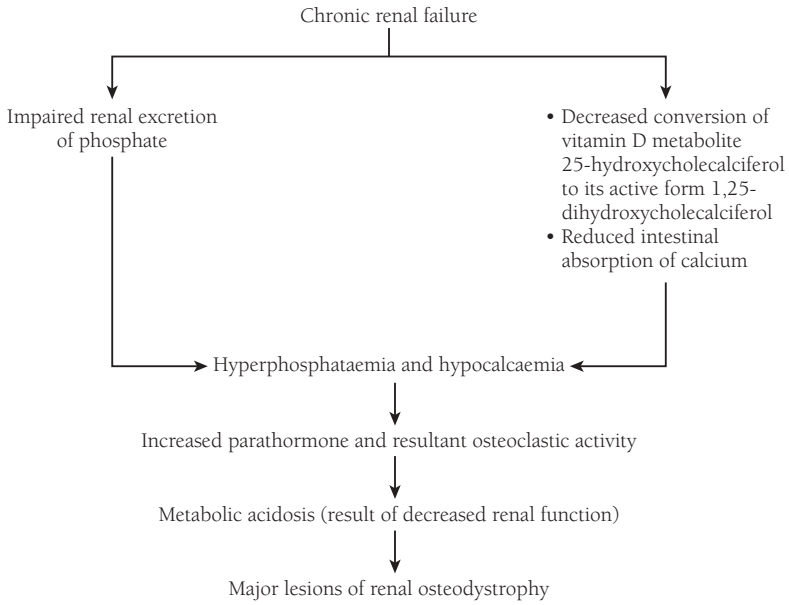
- Minor degree of generalized bone rarefaction to prominent areas of bone destruction (lytic lesion) with cyst formation
- Increased number of bizarre osteoclasts at the surface of moth-eaten trabeculae and cortex
- Replacement of bone and bone marrow by fibrosis
- In cancellous bone, osteoclasts tunnel in and dissect along the length of trabeculae to create the appearance of rail road tracks **(dissecting osteitis)**
- Microfractures and microhaemorrhages occur in the marrow cavity inducing an influx of macrophages and repair tissue. There is formation of masses of reactive tissue called **Brown tumours** (highly vascular tissue with abundant haemosiderin), which may undergo cystic degeneration (**osteitis fibrosa cystica** or **von Recklinghausen disease of** bone).

Q. Enlist the salient clinicopathological features of renal osteodystrophy.

Ans. ***Salient features of renal osteodystrophy (metabolic bone disease)*:**

- Encompasses a number of skeletal abnormalities appearing in patients of chronic renal failure or patients on long-term dialysis.
- More common in children.
- Clinical symptoms of bone disease in advanced renal failure appear in less than 10% of the patients.
- Radiological and histological changes are observed in fairly large proportion of cases.

Pathogenesis (Flowchart 21.3)



FLOWCHART 21.3. Clinicopathological features of renal osteodystrophy

Manifestations

- Osteomalacia in adults and rickets in children
- Secondary hyperparathyroidism and osteitis fibrosa cystica
- Osteosclerosis (enhanced bone density in the upper and lower margins of vertebrae)
- Metastatic calcification (in medium-sized blood vessels, periarticular tissue, myocardium, eyes, lungs and gastric mucosa)

Dialysis-Related Metabolic Bone Disease

Long-term dialysis employing an aluminium-containing solution is a major cause of metabolic bone lesions (aluminium interferes with deposition of calcium hydroxyapatite in bone and results in osteomalacia, secondary hyperparathyroidism and osteitis fibrosa cystica). Also, in such cases, accumulation of β_2-microglobulin amyloid causes dialysis-related amyloidosis.

Q. Describe in brief Paget disease of bone (osteitis deformans).

Ans. First described by Sir James Paget in 1877; Paget disease of bone is an osteolytic and sclerotic bone disease of uncertain aetiology. It has the following salient features:

- May involve one (monostotic) or more bones (polyostotic).
- Mainly affects males over the age of 50 years.
- Thought to be a slow virus infection caused by a paramyxovirus. The virus infested osteoclasts release IL-6 which induces osteoclastic recruitment leading to resorption of bone.

Clinical Features

- Monostotic Paget disease mainly involves the pelvis, femur, skull and vertebrae.
- Order of involvement in polyostotic Paget disease is: vertebrae, pelvis, femur, skull, sacrum and tibia.

- Monostotic form of the disease usually remains asymptomatic (discovered incidentally on radiological examination).
- Polyostotic form, however, is more widespread and may produce pain, fractures, skeletal deformities, bone overgrowth (**leontiasis ossea:** overgrowth of the craniofacial skeleton), and occasionally, sarcomatous transformation.

  May also manifest with platybasia (flattening of the base of skull due to weakened bone), chalkstick type of fractures in the long bones or severe secondary osteoarthritis with marked elevation of serum alkaline phosphatase and normal-to-high serum calcium level.

Pathology

Three sequential stages have been identified in Paget disease:

- **Initial osteolytic stage:** Large areas of osteoclastic resorption produced by increased number of osteoclasts are seen.
- **Mixed osteolytic–osteoblastic stage:** Imbalance between osteoblasts laying down new bone and osteoclastic resorption occurs so that mineralization of the newly laid matrix lags behind, resulting in development of a characteristic **mosaic pattern of osteoid seams** or **cement lines.**
- **Quiescent osteosclerotic stage:** After many years, excessive bone formation results so that the bone becomes more compact and dense, producing **osteosclerosis**. However, newly formed bone is poorly mineralized, soft and susceptible to fractures. **Radiologically**, this stage produces characteristic **cotton-wool appearance** of the affected bone.

Q. Classify fibro-osseous lesions of bone. Outline the salient clinicopathological features of its different types.

Ans. Fibro-osseous lesions of bone include

Fibrous Dysplasia (FD)

- **Benign condition**, possibly of **developmental origin** characterized by the presence of localized area of replacement of bone by fibroconnective tissue with a characteristic whorled pattern containing trabeculae of woven bone.
- **Radiologically**, the typical focus of FD is well-demarcated and has a **ground-glass appearance**.
- FD has three subtypes:

1. **Monostotic FD**
   (a) Monostotic FD usually affects a solitary bone.
   (b) It is the most common type of FD and comprises about 70% of all cases.
   (c) Most patients are between 20 and 30 years of age.
   (d) Bones most often affected, in descending order of frequency are ribs, cranio-facial bones (especially maxilla), femur, tibia and humerus.
   (e) The condition generally remains asymptomatic, and is discovered incidentally, but infrequently may produce tumour-like enlargement of the affected bone.
2. **Polyostotic FD**
   (a) This form of fibrous dysplasia comprises 25% of all patients and affects several bones.
   (b) Earlier onset than the monostotic form.
   (c) Most frequently affected bones are craniofacial bones, ribs, vertebrae and long bones of the limbs.
   (d) Spontaneous fractures and skeletal deformities are common in the childhood polyostotic form of the disease.
3. **Albright syndrome**
   (a) Also called **McCune–Albright syndrome**
   (b) A form of polyostotic FD associated with endocrine dysfunction
   (c) Accounts for less than 5% of all cases

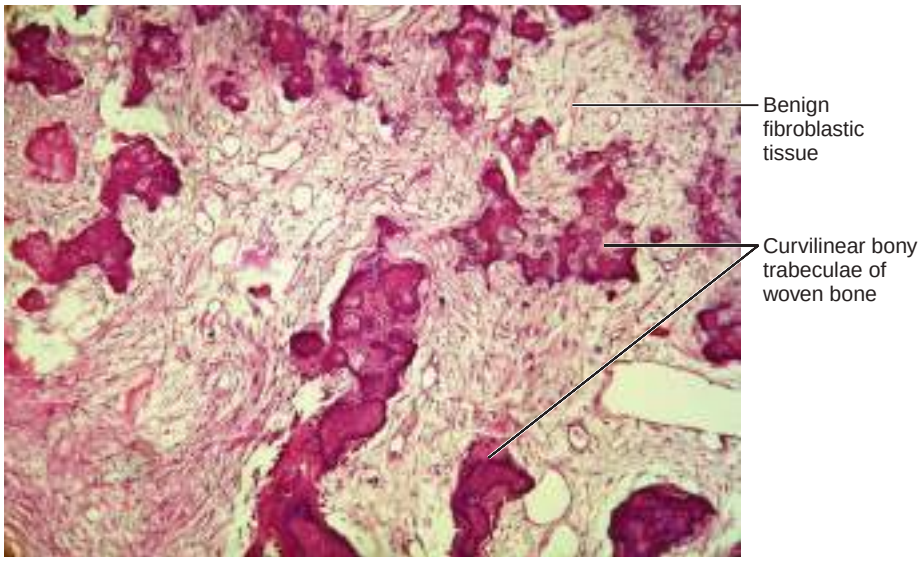


FIGURE 21.18. Section from FD composed of benign-looking fibroblastic tissue arranged in a loose, whorled pattern within which irregular and curved trabeculae of woven (nonlamellar) bone are laid down (H&E; 100×).

(d) More common in females
(e) Characterized by polyostotic bone lesions, skin pigmentation (café-au-lait macular spots), sexual precocity and infrequently other endocrinopathies.

Gross Pathology

- Lesions appear as sharply demarcated localized defects measuring 2–5 cm in diameter.
- Thin, smooth overlying cortex with the cut section of the lesion showing replacement of normal cancellous bone of the marrow cavity by gritty, grey-pink, rubbery soft tissue, which may have areas of haemorrhage, myxoid and cystic degeneration.

Microscopy (Fig. 21.18)

- Characteristic benign-looking fibroblastic tissue arranged in a loose, whorled pattern in which irregular and curved trabeculae of woven (nonlamellar) bones are laid down.
- Numerous osteoclasts in relation to bony trabeculae.
- Rarely, secondary malignant change, most often osteogenic sarcoma.

Fibrous Cortical Defect (Metaphyseal Fibrous Defect, Nonossifying Fibroma)

Salient Features

- Occurs in the metaphyseal cortex of long bones in children
- Most commonly involves tibia or femur
- Generally solitary, but may be multiple and bilaterally symmetrical

X-Ray

Eccentric lesion in the metaphysis with a sharply delimited border

Pathogenesis

Possible hypothesis:

- Arises as a result of some developmental defects involving the epiphyseal plate
- Could be a tumour of histiocytic origin (based on close resemblance to fibrohistiocytic tumours)

Gross Pathology

- Lesion is generally small, less than 4 cm in diameter, granular and brown.
- Larger lesion (5–10 cm) referred to as **nonossifying fibroma**.

Microscopy

- Cellular masses of fibrous tissue showing storiform pattern interspersed with numerous multinucleate osteoclast-like giant cells.
- Focal areas showing haemosiderin-laden macrophages and foam cells.

JOINTS

Functions of Joints

- Enable movement and provide mechanical support
- Solid joints provide structural integrity
- Cavitated joints are lined by synovial cells and aid in movement

Components of Synovial Lining

- Type A cells: Macrophage-like; synthesize hyaluronic acid
- Type B cells: Fibroblast-like; produce various proteins

Functions of Articular Cartilage

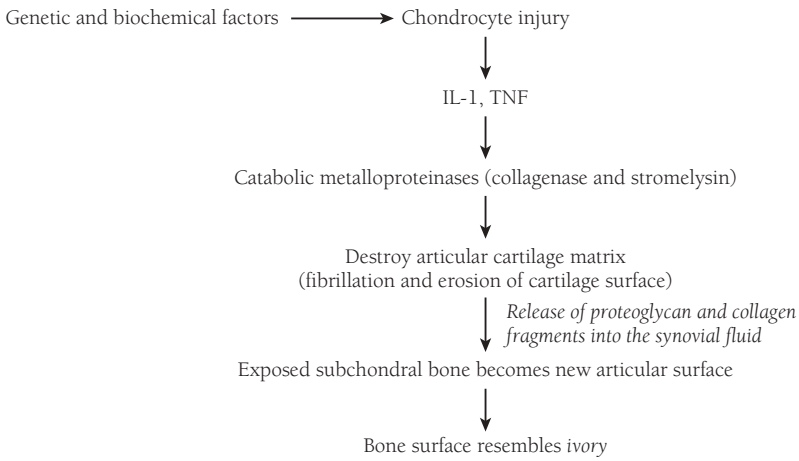
- Friction-free movement in joints
- Spreading the load evenly in weight-bearing joints, so that the underlying bones absorb shock and weight without being crushed.

Q. Outline the aetiopathogenesis, clinical and pathological features of osteoarthritis (OA).

Ans. OA or degenerative joint disease is characterized by age and mechanical stress dependent progressive erosion of articular cartilage. It is more common in females than in males and may be primary or secondary in origin. Secondary osteoarthritis occurs following metabolic disorders (ochronosis, haemochromatosis), deformity, trauma, fracture, obesity, severe mechanical stress and diabetes.

Clinical Features

- OA primarily targets weight-bearing joints (hip, knee, distal interphalangeal joints of hands and lower lumbar vertebrae).
- May be asymptomatic or presents with the following manifestations:
  - Deep aching pain which worsens with movement
  - Stiffness and limitation of movement with crepitus (crackling sound)
  - Bone eburnation (when cartilaginous protection is reduced, subchondral bone may be exposed and damaged. This is followed by regrowth leading to a proliferation of ivory-like, dense, reactive bone in central areas of cartilage)
  - Small fractures in articular bones
  - Atrophy of regional muscles and laxity of ligaments (consequent to decreased movement because of the pain)
  - Degenerative changes (result in formation of hard, bony, painless enlargements called *Heberden's nodes* at the base of distal interphalangeal joint of the fingers and *Bouchard's nodes* on the proximal interphalangeal joints).
- Reactive bone formation at the margins of the joints (osteophytes). Osteophytes in spine may cause compression of cervical/lumbar nerve roots causing pain, muscle spasms and neurological deficit.



FLOWCHART 21.4. Pathogenesis of osteoarthritis

Pathogenesis (Flowchart 21.4)

Genetic and biochemical factors lead to chondrocytes injury. In early OA, chondrocytes proliferate and release inflammatory mediators which result in injury to the synovium and subchondral bone. In late OA, repeated/persistent inflammation leads to chondrocytes drop out, loss of cartilage and subchondral bone alterations.

Diagnosis

There is no specific laboratory test for osteoarthritis, no means of preventing primary osteoarthritis and no definite methods for arresting its progression.

Q. Outline the aetiopathogenesis and clinicopathological features of rheumatoid arthritis (RA).

Ans. RA is a chronic, nonsuppurative, autoimmune disease.

Clinical Features

- It is a multisystem disease which involves three or more joints and has an insidious onset.
- Occurs between 40 and 70 years of age and shows female preponderance.
- Commonly affected joints include metacarpophalangeal and proximal interphalangeal joints of hands along with larger joints like wrists, elbows, ankles and knees.
- Morning stiffness lasting longer than 1 h before improvement is a classic feature (unlike osteoarthritis in which the pain and stiffness gets worse with progressive use of the joint during the day).
- Advanced disease in the hands and wrists produces ulnar deviation of the fingers (**Swan neck deformity**) and may lead to laxity of the soft tissues.
- **Carpal tunnel syndrome** (compression of median nerve) is a common manifestation.
- Also seen are general malaise, weakness, fever of unknown origin, weight loss, myalgias and inflammation of tendons and bursae.

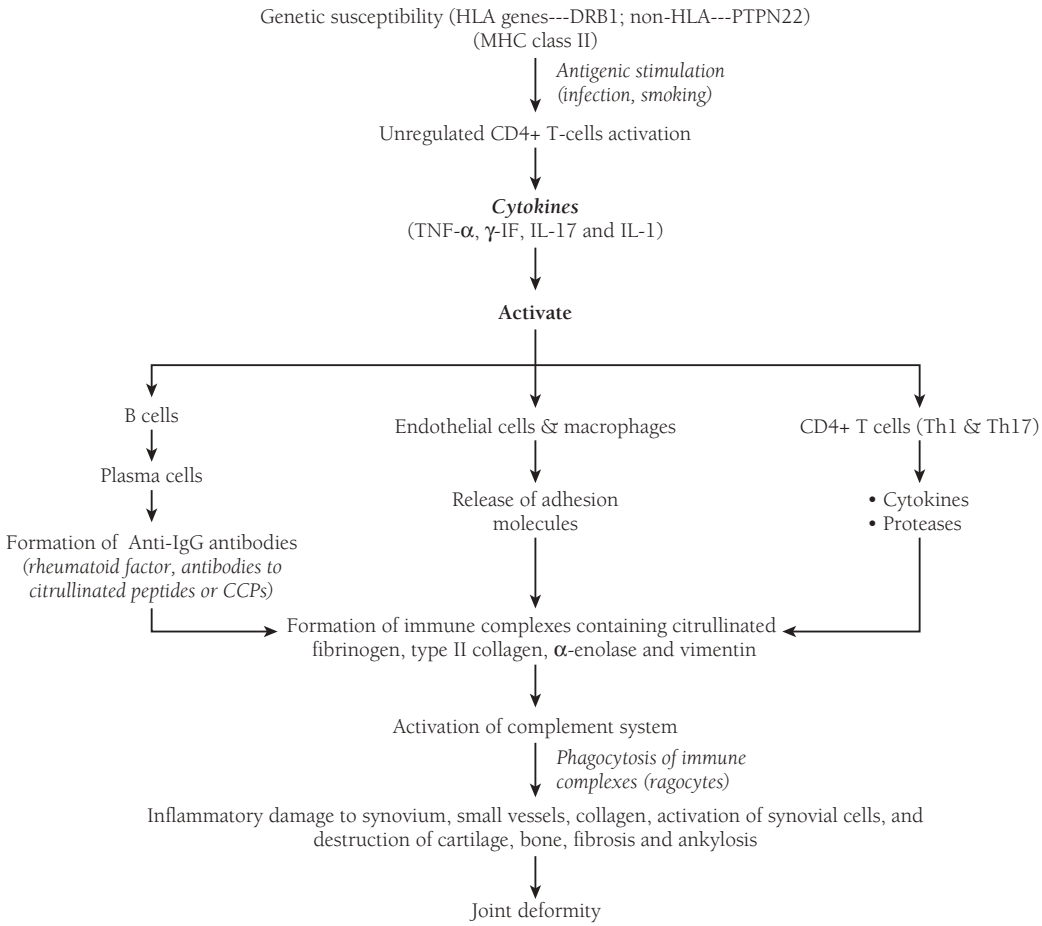
Extra-Articular Manifestations of RA

- Subcutaneous (rheumatoid) nodules, which occur on extensor surface of limbs, occiput and sacrum
- Cardiac involvement (carditis) and medium-to-small vessel vasculitis
- Skin ulceration, gangrene and obliterative endarteritis (associated with high titres of RA)

- Peripheral neuropathy
- Pulmonary involvement (Pleuritis, intrapulmonary nodules, interstitial fibrosis in the form of Caplan syndrome—restrictive lung disease with rheumatoid nodules and coal worker's pneumoconiosis)
- Hepatitis
- Ocular involvement (scleritis, episcleritis and dryness of the eye)
- Secondary amyloidosis and Sjögren syndrome

Pathogenesis

Pathogenesis of RA has been depicted in Flowchart 21.5.



FLOWCHART 21.5. Pathogenesis of rheumatoid arthritis

Rheumatoid factor (RF) is formed as a result of local stimulation of B cells, which produces IgM autoantibodies directed against the Fc receptor for IgG.

Morphology

- Synovial hyperplasia (multilayering of synovial cells)
- Infiltration of synovium by dense perivascular inflammatory infiltrate composed of B cells and CD4+ T cells (at places forming lymphoid aggregates), plasma cells and macrophages
- Increased vascularity due to vasodilatation and telangiectasia
- Deposition of fibrin in synovium and accumulation of neutrophils in synovial fluid

- Osteoclastic activity in underlying bone
- Pannus formation (pannus is a combination of neovascularization, inflammation and fibrinoid deposits, which progressively destroys the underlying cartilage and subchondral bone)

X-Ray

- Joint effusion
- Juxta-articular osteopenia with bone erosions and narrowing of joint spaces due to loss of articular cartilage

Diagnosis

At least four of the following should be present for the diagnosis of RA:

- Morning stiffness of 1 h for at least 6 weeks
- Arthritis and soft tissue swelling of 3 joints, present for at least 6 weeks
- Arthritis of hand joints, present for at least 6 weeks
- Symmetric arthritis, present for at least 6 weeks
- Subcutaneous nodules in specific places
- Rheumatoid factor above the 95th percentile
- Radiological changes suggestive of joint erosion

Q. Enlist the salient clinicopathological features of juvenile rheumatoid arthritis (JRA).

Ans. Salient features of JRA:

- It is a chronic inflammatory condition that begins in patients under 16 years of age.
- Manifests with abrupt onset of spiking fever, transient skin rash, hepatosplenomegaly and serositis and affects knees, wrists, elbows and ankles (large joints affected more than small joints).
- It is typically RA-negative, rheumatoid nodule-negative and ANA-positive.

Q. What is infectious arthritis? Enlist its important clinicopathological features.

Ans. Salient features of infectious arthritis:

- Infectious arthritis is defined as arthritis caused by infection with a microbial pathogen.
- May occur secondary to haematogenous spread, osteomyelitis and as a complication of intra-articular infection or surgery.
- Common causative organisms are *gonococci, meningococci, pneumococci, staphylococci, streptococci, H. influenza,* Gram-negative bacilli and *M. tuberculosis*.
- Patient presents with sudden onset of pain, swollen joints, restricted mobility, fever, leucocytosis and increased ESR. Commonly involved joints include knees, hips, shoulders, elbows and wrists.
- Predisposing conditions include immune deficiency, debilitating illness, joint trauma and intravenous drug abuse.
- *Tuberculous arthritis* is a chronic progressive monoarticular arthritis, which mainly affects the weight-bearing joints, eg, hips, knees and ankles. Systemic symptoms may or may not be present.

Q. Enumerate the various crystallopathies.

Ans. Articular crystal deposits include

- **Endogenous crystals:** Monosodium urate (MSU), calcium pyrophosphate dihydrate and calcium phosphate
- **Exogenous crystals:** Corticosteroids, talc, polyethylene and silicone

Q. Write briefly on gouty arthritis.

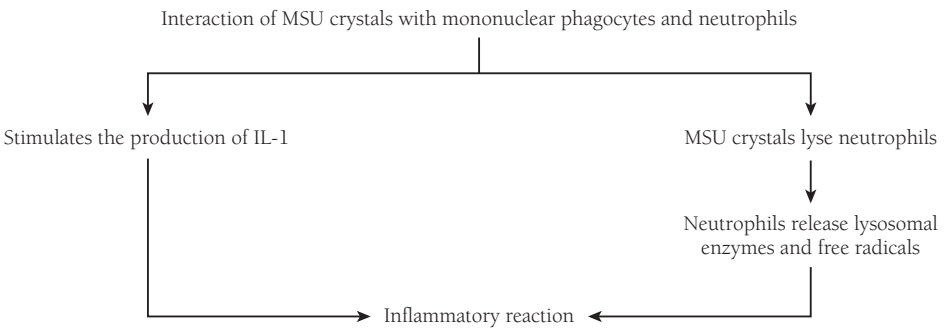
Ans. Salient features of gouty arthritis:

- Gouty arthritis is a male dominant disease, characterized by hyperuricaemia with plasma urate level more than 7 mg/dL (upper limit of solubility of MSU in serum at 37°C and blood pH).
- It involves the metatarsophalangeal joints followed by ankles and knees; wrists may be affected later.
- Typical manifestations include recurrent attacks of acute arthritis and deposits of MSU tophi (meaning *porous stones*) in soft tissue and renal disease affecting interstitium and blood vessels (uric acid nephrolithiasis).

Types

- **Metabolic gout (10% cases):** Due to disorder in metabolism of uric acid (a product of purine metabolism) leading to its overproduction.
- **Renal gout (90% cases):** Due to reduced renal excretion of uric acid secondary to diabetes mellitus, leukaemia, diuretic therapy, treatment of disseminated cancer and drugs like aspirin, pyrazinamide, nicotinic acid, ethambutol and ethanol.

Pathogenesis (Flowchart 21.6)



FLOWCHART 21.6. Pathogenesis of gouty arthritis

X-Ray

Juxta-articular bone erosion by crystal deposits with loss of joint space.

Pathology

- **Acute gout:** Predominantly a disease of lower extremities, acute gout most commonly affects the great toe. It is triggered by precipitation of needle-shaped crystals of MSU from serum or synovial fluid, which leads to an intensely painful joint effusion containing crystals, polymorphs and macrophages, lasting for hours to week.
- **Chronic gout:** This is characterized by presence of tophi, which usually develop after approximately 10 years of disease. Tophi represent deposits of MSU that occur in tissue most commonly in and around the affected joints. Sections through a tophus exhibit an exuberant granulomatous reaction complete with foreign body multinucleated giant cells surrounding a central core of amorphous MSU crystals.
- **Pseudogout:** Characterized by deposition of calcium pyrophosphate dihydrate crystals in the joint cavities, pseudogout can be sporadic (idiopathic), hereditary or secondary. The knee joint is affected in more than 50% cases and the age of the patients is more than 50 years. The crystals are rhomboid in shape and deposit in the articular cartilage (chondrocalcinosis). It may be asymptomatic or manifest as acute, subacute or chronic arthritis.

MUSCLE

Muscle Terminology

- Myofibre or myocyte: a muscle cell
- Sarcolemma: the plasma membrane of a muscle cell
- Sarcoplasm: the cytoplasm of the muscle cell
- Sarcoplasmic reticulum: the endoplasmic reticulum of a muscle cell
- Sarcosome: the mitochondria of a muscle cell
- Sarcomere: the contractile or functional unit of muscle

The entire muscle is surrounded by connective tissue called the **epimysium** and is made up of smaller bundles known as **fascicles**. Fascicles are actually bundles of individual muscle cells **(myofibres or myocytes)**. Each of these bundles is surrounded by a connective tissue sheath called the **perimysium**. Each muscle cell is surrounded by a connective tissue sheath known as the **endomysium**. This sheath is very important in the physiology of muscle contraction because it electrically insulates the individual muscle cells from each other. At the ends of the muscle, all of the connective tissue sheaths (epimysium, perimysium and endomysium) converge to form a tendon, which will connect the muscle to its attachment site.

Each muscle fibre (muscle cell) contains all of the organelles that we find in other cell types. Although these organelles are the same as in other cells, they are given special names (the prefixes 'sarco' and 'myo' both refer to muscle). The nucleus contains the genetic material of the muscle cell. **Sarcolemma** is the name given to the plasma membrane of the muscle cell. The **Cytosol** is the cytoplasm of the muscle cell.

The **sarcoplasmic reticulum** is the endoplasmic reticulum of the muscle cell. There are sac-like regions of the sarcoplasmic reticulum known as **terminal cisternae**. The terminal cisternae act as calcium storage sites. The calcium ions stored in the terminal cisternae are essential in muscle contraction. A myofibril is a cylindrical bundle of contractile proteins found within the muscle cell. Myofibrils are composed of individual contractile proteins called **myofilaments**. These myofilaments are generally divided into thick and thin myofilaments. The thin myofilaments are composed mainly of a protein known as actin. Actin filaments are anchored into the Z line of a sarcomere. The thick myofilaments are composed mainly of the protein myosin.

It is the orderly overlapping of the actin and myosin filaments that give cardiac and skeletal muscle their striated appearance (light and dark bands).

Each muscle is supplied by a motor nerve originating from neurons in the spinal cord or brain stem.

Types of Fibres in Human Skeletal Muscle

- **Type I** are slow twitch fibres (red muscle) that are rich in mitochondria and oxidative enzymes and have a great capacity for long, sustained contraction without fatigue (eg, soleus muscle).
- **Type II** are fast twitch fibres (white muscle) that are poor in mitochondria, use both oxidative metabolism and anaerobic glycolysis and are quicker to fatigue (eg, biceps muscle).

Reactions of Muscle Fibres

- Segmental necrosis (only a portion of myocyte is destroyed, undergoes myophagocytosis and replacement by collagen and fat)
- Vacuolization, alteration in structural proteins and accumulation of intracytoplasmic deposits
- Regeneration
- Hypertrophy

Q. Classify disorders of muscle.

Ans. Muscle disorders include

1. **Muscle weakness** secondary to diseases involving the motor neuron pathways, neuromuscular synapse (eg, myasthenia gravis).

2. **Neurogenic atrophy** occurs when a motor neuron or its axon degenerates, leading to atrophy of both Type I and Type II fibres.
3. **Muscular dystrophy (MD)** is an inherited progressive primary muscle disease that most commonly presents in early childhood.
4. **Congenital myopathies** are rare, primary, nonprogressive muscle diseases that present at birth with poor muscle tone (eg, central core disease, nemaline [rod] myopathy).
5. **Polymyositis** and **dermatomyositis** are connective tissue disorders which involve muscle.

Q. Classify and describe muscular dystrophies.

Ans. Muscular dystrophies include Duchenne and Becker dystrophy (X-linked inheritance), limb girdle dystrophy (autosomal recessive inheritance), fascioscapulohumeral and oculopharyngeal dystrophy (autosomal dominant inheritance). The most common types have been described below:

(a) **Duchenne muscular dystrophy (DMD)**
- Most severe and most common type of dystrophy
- X-linked inheritance; females are carriers
- Presents with progressive muscle weakness and wasting, which manifests by 5 years
- Paralysis and death by second to third decade
- Weakness begins in the pelvic girdle muscles, and then shoulder girdle is affected.
- Characterized by a positive Gower manoeuvre (requiring the assistance of upper extremities to stand up) and pseudohypertrophy (enlargement of calf muscles with weakness)
- May effect cardiac muscle resulting in arrhythmias and heart failure
- May be associated with intellectual impairment
- Death results from respiratory insufficiency, pulmonary infection and cardiac failure

***Pathogenesis*:**
- Abnormalities in a gene encoding dystrophin located in the Xp21 region
- Deletion (most common), point mutation also seen
- Dystrophin and dystrophin-associated protein complex anchors actin to membrane glycoprotein; absence of dystrophin causes transfer of the force of contraction to connective tissue and myocyte degeneration

(b) **Becker muscular dystrophy (BMD)**
- Less common; less severe
- BMD patients have mutations in dystrophin gene resulting in decreased amount of dystrophin, usually of abnormal molecular weight.

Morphology of Dystrophic Muscle

Most histopathological abnormalities are common to DMD and BMD and include

- Variation in fibre size (presence of both small and large fibres) with fibre splitting
- Increased number of internalized nuclei (normally less than 3% are internalized)
- Degeneration, necrosis and phagocytosis of muscle fibres
- Regeneration of muscle fibres and proliferation of endomysial connective tissue. DMD cases also show enlarged, rounded hyaline fibres with loss of cross striations (hypercontracted fibres); a finding rare in BMD.

Laboratory Findings

- Antenatal diagnosis of dystrophin defect using recombinant DNA technology
- Serum CK (creatinine kinase) levels decline as muscle tissue is progressively replaced by fat and fibrous tissue.

Q. Write briefly on myasthenia gravis.

Ans. Autoimmune disease characterized by reduction in acetylcholine receptors due to the presence of an autoantibody against them.

- Acetylcholine receptor antibody accelerates degradation of the receptor aided by complement activation and blocks receptor function.
- Myasthenia gravis may be associated with thymic hyperplasia as well as thymomas.
- Ptosis and diplopia are the most common initial presentations.
- Histopathology is not diagnostic; Type II fibre atrophy may be observed in late stage.
- Treatment includes anticholinesterase drugs, thymectomy, immunosuppression and plasmapheresis.

Q. Write briefly on Lambert–Eaton myasthenic syndrome.

Ans. Develops as a paraneoplastic process, commonly with small cell carcinoma of lung.

- Patients develop proximal muscle weakness with autonomic dysfunction.
- No improvement found with anticholinesterase agents.
- Content of anticholinesterase is normal in neuromuscular junction synaptic vesicles, but fewer vesicles are released.

Soft Tissue

Q. Summarize the clinicopathological features of soft tissue tumours.

Ans. Clinicopathological features of soft tissue tumours are summarized in Table 22.2.

TABLE 21.2. Clinicopathological features of soft tissue tumours

Tumour type	Distribution	Salient features
Lipomatous tumours		
Lipoma	Trunk, neck, proximal extremities	**Most common benign soft tissue tumour.** Arises in subcutaneous tissue. Conventional lipoma is a well-encapsulated mass of mature adipocytes. **Generalized lipomatosis (Dercum disease):** Multiple lipomas in subcutaneous tissue, which on rare occasions, may transform into liposarcoma. Other variants include fibrolipoma, angiolipoma, spindle cell lipoma, myelolipoma and pleomorphic lipoma.
Fibrous tumours		
Fibrosarcomas	Thigh, upper limb, retroperitoneum	Unencapsulated, infiltrative, fleshy masses, varying from slow-growing lesions, which are better differentiated to cellular lesions characterized by a 'herringbone' (interlacing) pattern. Have 40–50%, 5-year survival rate. May arise secondary to irradiation.
Fibrohistiocytic tumours		
Benign fibrous histiocytoma	Lower extremities	Solitary, slow growing, unencapsulated, reddish nodule. Overlying epidermis may show hyperplasia. Benign proliferation of spindle cells confined to the dermis and subcutis. Cells are arranged in a storiform pattern and may show foam cells, haemosiderin and multinucleate giant cells. Tumours arising from the dermis are called **dermatofibromas.** Other benign fibrohistiocytic tumours include **juvenile xanthogranuloma, epithelioid histiocytoma** and **reticulohistiocytoma.**
Dermatofibrosarcoma protuberans	Chest wall, trunk	Low-grade malignant dermal tumour that may show overlying ulceration. Characteristic 'cartwheel' pattern of spindle cells with increased mitotic activity and numerous giant cells.

Continued

TABLE 21.2. Clinicopathological features of soft tissue tumours—cont'd

Tumour type	Distribution	Salient features
Malignant fibrous histiocytoma	Extremities, retroperitoneum	Most common soft tissue sarcoma; affects older adults (fifth to sixth decade).
Skeletal muscle tumours		
Rhabdomyoma	Heart	Associated with tuberous sclerosis (AD inheritance).
Rhabdomyosarcomas		Most common sarcoma in children and most common striated muscle malignancy.
Embryonal rhabdomyosarcoma	Head and neck, vagina, paratesticular region, bladder	Most common type of rhabdomyosarcoma. Botryoid type presents as grape-like mass protruding from the walls of hollow mucosa-lined structures (vagina or male urethra). Rhabdomyoblasts have cross-striations and stain-positive for desmin. Embryonal RMS may range from highly differentiated neoplasms containing rhabdomyoblasts with large amounts of eosinophilic cytoplasm and cross-striations to those with poorly differentiated tumour cells.
Alveolar rhabdomyosarcoma	Distal extremities	Occurs between 10 and 25 years of age. Second most common type of skeletal muscle malignancy and has the worst prognosis. Fibrous septae divide the cells into clusters. Cells in the centre are discohesive, while those at the periphery adhere to the septae giving rise to an alveolar pattern.
Pleomorphic rhabdomyosarcoma	Deep soft tissue of adults	Composed of numerous large, sometimes multinucleated pleomorphic tumour cells. Least common type of skeletal muscle malignancy.
Smooth muscle tumours		
Leiomyoma	Uterus (myometrium), genitals, skin (erector muscle), extremities, retroperitoneum, most common benign GI tumour	Most common tumour in women. Composed of fascicles of spindle cells that intersect each other at right angles (whorled appearance). Have blunt-ended cigar-shaped nuclei. Rarely progress to leiomyosarcoma.
Leiomyosarcoma	Extremities, retroperitoneum	Most commonly arises from wall of blood vessels. Increased mitotic count and atypical mitoses differentiate it from a cellular leiomyoma. **Most common sarcoma in the GI tract and uterus.** Composed of fascicles of malignant spindle-shaped cells with cigar-shaped nuclei.
Neural tumours		
Benign nerve sheath tumours	Skin, peripheral nerves	Arise sporadically or in association with neurofibromatosis type I. Well-circumscribed unencapsulated lesions composed of spindle-shaped cells with wavy nuclei. Stroma may be collagenized to myxoid.
Plexiform neurofibroma	Major nerve trunks	Most arise in conjunction with type I neurofibromatosis. Nerve irregularly expanded.
Malignant peripheral nerve sheath tumour (MPNST)	Major nerve trunks (sciatic)	Arise *de novo* or from transformation of a plexiform neurofibroma. Strong association with NF I. Poorly defined infiltrative tumour mass composed of spindled cells with elongated wavy nuclei showing extreme pleomorphism. Mitoses and necrosis are common.
Tumours of unknown origin		
Synovial sarcoma	Extremities	Misnomer; not derived from synovial tissue. Less than 10% intra-articular. Located around rather than in the joint. Male predominance with peak incidence between 25 and 35 years of age. They may be monophasic or biphasic. Monophasic tumours are composed of only spindled cells or rarely only epithelial cells, whereas biphasic tumours are composed of both, with the epithelial cells arranged in a gland-like pattern. Most synovial sarcomas are associated with translocation t(x;18)(p11;q11) producing SS18-SSX1.

22

The Skin

Normal skin is composed of different cell types, namely:

- **Squamous epithelial cells or keratinocytes** (produce keratin, defensins and cytokines responsible for regulation of proliferation and differentiation of adjacent epidermal cells, as well as cells in the dermis).
- **Melanocytes** (responsible for production of melanin).
- **Langerhans cells** (epidermal dendritic cells that process antigens).
- **Merkel cells** (reside within the basal layer and function as the neuroendocrine cells of the skin).

Layers of Skin

1. **Epidermis** - Composed of stratified squamous epithelium with the following layers:
   (a) Stratum basale: Contains actively dividing stem cells along the basement membrane. As the basal cells divide, daughter cells migrate upwards.
   (b) Stratum spinosum: Intercellular bridges called desmosomes link the cells together. The cells are polygonal and become increasingly flattened as they move upwards.
   (c) Stratum granulosum: Constituted by 1–3 layers of flat cells with keratohyalin basophilic granules.
   (d) Stratum corneum: Contains anucleate cells with keratin.
2. **Dermis** - Consists of two parts:
   (a) Superficial papillary dermis
   (b) Deep reticular dermis

Dermis contains specialized appendages called adnexal structures, eg, hair. Hair follicles produce hair shafts and are closely associated with sebaceous (oil-producing) glands and erector pilaris muscle. Sweat glands guard against the deleterious effects of temperature variations.

Definitions of Macroscopic Terms

- **Macule:** Circumscribed flat lesion up to 5 mm in diameter, distinguished from the surrounding skin by its coloration, without any alteration in the texture of the skin
- **Patch:** Circumscribed flat lesion more than 5 mm in diameter, distinguished from the surrounding skin by its coloration
- **Papule:** Circumcised solid, dome-shaped or flat-topped lesions, 5 mm or less in size
- **Nodule**: Solid, raised and bumpy lesion with spherical contour greater than 5 mm
- **Plaque:** Elevated, flat-topped lesion greater than 5 mm across
- **Vesicle:** Fluid-filled, raised lesion 5 mm or less across
- **Bulla:** Fluid-filled, raised lesion more than 5 mm across
- **Blister:** Common term used for vesicle or bulla
- **Pustule:** Discrete pus-filled lesion
- **Wheal:** Itchy, transient, elevated lesion formed as a result of dermal oedema
- **Scale:** Dry, horny, plate-like excrescence due to imperfect cornification
- **Lichenification:** Thickened and rough skin with prominent skin markings, usually a result of frequent rubbing
- **Excoriation:** Raw, linear lesion due to breakage of epidermis, subsequent to trauma

Definitions of Microscopic Terms

- **Hyperkeratosis:** Thickening of stratum corneum, which may be associated with qualitative abnormalities of keratin
- **Parakeratosis:** Retention of nuclei in the stratum corneum
- **Hypergranulosis:** Hyperplasia of stratum granulosum
- **Acanthosis:** Diffuse epidermal hyperplasia
- **Papillomatosis:** Surface elevation caused by hyperplasia and enlargement of contiguous dermal papillae
- **Dyskeratosis:** Abnormal or premature keratinization within cells below stratum granulosum
- **Spongiosis:** Intercellular oedema of epidermis
- **Acantholysis:** Loss of intercellular junctions resulting in loss of cohesion between keratinocytes
- **Hydropic swelling:** Intracellular oedema seen in keratinocytes
- **Exocytosis:** Infiltration of the epidermis by inflammatory or circulating blood cells
- **Erosion:** Discontinuity of the skin or incomplete loss of the epidermis
- **Ulceration:** Discontinuity of the skin or incomplete loss of the epidermis
- **Lentiginous:** Linear pattern of melanocyte proliferation within the epidermal basal layer

Q. Define dermatitis.

Ans. Dermatitis is a nonspecific term, which indicates 'inflammation of the skin'.

Q. Write briefly on eczematous dermatitis.

Ans. Eczema (spongiotic dermatitis) is characterized by a large group of pruritic skin lesions with different aetiologies. Eczema has three stages: **acute, subacute** and **chronic.**

1. **Acute eczema** is characterized by oozing, crusting and erythematous papulovesicular eruptions with spongiosis (intercellular oedema) in the epidermis.
2. **Subacute eczema** is associated with crusts developing over ruptured vesicles in the stratum corneum.
3. **Chronic eczema** shows raised scaly plaques or lichenification (thickening due to hyperkeratosis from constant scratching) and hyperpigmentation.

Eczematous dermatitis is classified into the following:

1. **Atopic dermatitis** is a Type I IgE-mediated disease that presents in neonates as a rash on the cheeks, trunk and extensor surfaces, and moves to the flexor creases as the child grows older.
2. **Allergic contact dermatitis** is a Type IV cell-mediated hypersensitivity reaction against poison ivy, oak, nickel and chemicals found in the household cleaners, cosmetics, fabrics, dyes, medications and rubber products.
3. **Irritant contact dermatitis,** the most common type of eczematous dermatitis, is a nonimmunologic reaction due to the local toxic effect of a chemical on the skin (eg, detergents in soaps).
4. **Photoeczematous dermatitis** is a type of allergic contact dermatitis that is caused by ultraviolet (UV) light reacting with drugs that have a photosensitizing effect (eg, tetracycline, sulphonamides and thiazides).
5. **Drug-related eczematous dermatitis:** Reaction to an internal circulating antigen derived from an ingested drug.

***Morphology*:**

Spongiosis is defined as the accumulation of oedema fluid within the epidermis. Intercellular bridges are stretched and become more prominent, giving rise to a spongy appearance. This is accompanied by superficial perivascular lymphocytic infiltrate and papillary dermal oedema.

Note: There are no specific histopathological features to distinguish various causes of eczema.

Q. Write briefly on superficial mycosis.

Ans. The superficial mycosis causing fungi (dermatophytes) make up a group of fungi that is confined to the outermost layers of the skin or its appendages.

- Tinea capitis is most common in children, in whom it presents with circular or ring-shaped patches (thus also called 'ringworm') of alopecia (hair loss) with erythema and scaling and is usually caused by *Trichophyton tonsurans*.
- *T. rubrum* and *T. mentagrophytes* are responsible for many other types of Tinea infections (eg, Tinea cruris, pedis and corporis).
- Tinea versicolor is caused by *Malassezia furfur* (a yeast) and is associated with areas of hyper- and hypopigmentation. Scrapings reveal the classic '**spaghetti (hyphae)** and **meatball (yeast)**' appearance.
- *Candida albicans* commonly produces disease involving the skin (common cause of diaper rash) and nails (onychomycosis).

Q. Enumerate and describe chronic inflammatory dermatoses.

Ans. Chronic inflammatory dermatoses include

1. **Psoriasis**
   - This is a chronic inflammatory dermatosis associated with arthritis, myopathy, enteropathy and heart disease
   - It affects skin of the elbows, knees, scalp and lumbosacral areas.
   - The most typical lesion is a well-demarcated, itchy, pink to salmon plaque covered by loosely adherent silvery white scales.
   - Nail changes are seen in up to 30% cases and include pitting, thickening, yellow-brown discoloration, crumbling and separation of the nail plate from the underlying bed (**onycholysis).**
   - Many of these patients present with psoriatic arthritis, which may manifest as classic distal interphalangeal joint involvement, symmetric polyarthritis, asymmetric oligoarthritis (the most common type of psoriatic arthritis) or as ankylosing spondylitis.
- Pathogenesis of psoriasis is multifactorial in origin with the contribution from the following:
  - Immunologic status of the individual
  - Genetic susceptibility (strong association with HLA-C especially with HLA-Cw*0602 allele)
  - Environmental factors
  - There is accumulation of $CD4^+$ T_H1 and $CD8^+$ T cells in the epidermis, which secrete cytokines and growth factors inducing keratinocyte hyperproliferation resulting in the characteristic lesions. It can be induced in susceptible individuals by local trauma **(Koebner phenomenon).**

  ***Morphology*:**
  - Marked epidermal thickening **(acanthosis)**
  - Regular downward elongation of rete ridges **(psoriasiform hyperplasia)**
  - Rapid epidermal cell turnover results in **loss of stratum granulosum with extensive parakeratotic scaling**
  - **Suprapapillary thinning** (thinning of the epidermal cell layer overlying the tips of dermal papillae)
  - Vessels bleed on removal of the scale, giving rise to multiple bleeding points **(Auspitz's sign)**
  - Neutrophils form small aggregates within the spongiotic superficial epidermis **(pustules of Kogoj)** and the parakeratotic stratum corneum **(Munro microabscesses)**
2. **Lichen planus**
   - Lichen planus is characterized by pruritic, purple, polygonal, papules and plaques in the skin and mucosa.
   - It is self-limited and may resolve spontaneously; oral lesions may persist for years.
   - The papules are highlighted by white dots or lines called **Wickham's striae.**
   - Pathogenesis is unknown, however, it is hypothesized that it occurs due to cytotoxic T cell response to an altered antigen in the basal cells.

   ***Morphology*:**
   - **Basal keratinocytes** show degeneration and necrosis.

- Anucleate necrotic basal cells called **colloid** or **Civatte bodies** are seen in the papillary dermis.
- There is epidermal hyperplasia, hypergranulosis and hyperkeratosis.
- **Interface dermatitis** (dense continuous band-like infiltrate of lymphocytes along the dermoepidermal junction) is classically seen. The infiltrate may assume an angulated zig-zag contour (saw toothing).

3. **Lichen simplex chronicus (LSC)**
   - LSC is characterized by roughening and gradual thickening of skin called lichenification (like lichen on a tree), due to repeated trauma or irritation (rubbing and scratching).
   - Sometimes the thickening may result in formation of nodules called **prurigo nodularis.**

   ***Morphology*:**
   - Acanthosis, hyperkeratosis and hypergranulosis.
   - Elongation of rete ridges, papillary dermal fibrosis and chronic dermal inflammatory infiltrate.

Q. Write briefly on verrucae.

Ans. Verrucae are common lesions of children and adolescents but may be encountered at any age.

- They are caused by low-risk types of human papilloma virus (HPV)
- Transmission is by direct contact and autoinoculation
- May regress spontaneously within 6 months to 2 years
- **Verruca vulgaris** is the most common type of wart (found frequently on dorsum of the hands, and periungual areas, seen as grey-white to tan, flat to convex papules with a pebble-like appearance).
- **Verruca plana or flat wart** is common on the face and dorsum of the hands, seen as smooth tan macules.
- **Verruca plantaris** and **palmaris** occur on the soles and palms, respectively, and are seen as rough, scaly lesions, 1–2 cm in diameter.
- **Condyloma acuminatum** occurs on the penis, female genitalia, urethra and perianal areas.

***Morphology*:**
- Verrucous epidermal hyperplasia and papillomatosis
- Viral cytopathic effect (haloes surrounding the infected nuclei)
- Infected cells show prominent keratohyalin granules

Q. Write briefly on blistering or bullous disorders.

Ans. Group of disorders in which blisters are the primary and the most distinguished feature:

1. **Pemphigus**
   - Rare, autoimmune blistering disorder resulting from loss of normal intercellular attachments
   - Three major variants:
     - Pemphigus vulgaris
     - Pemphigus foliaceus
     - Paraneoplastic pemphigus (pemphigus associated with internal malignancy)

   ***Pathogenesis*:**
   - **Pemphigus vulgaris** and **foliaceus** are caused by a type II hypersensitivity reaction (antibody directed against a fixed-tissue antigen) and are linked to specific HLA types.
   - Patient's sera contain pathogenic IgG antibodies to intercellular desmosomal proteins (desmoglein Types I and III).
   - **Pemphigus vulgaris** (most common type) involves mucosa and skin of scalp, face, axillae, groin, trunk and points of pressure.
   - **Pemphigus foliaceus** results in bullae confined to skin with infrequent involvement of mucosa.

***Morphology*:**
- Histological hallmark in all forms of pemphigus is **acantholysis** (separation of individual keratinocytes due to lysis of intercellular adhesion sites); detached acantholytic cells become rounded.
- In **pemphigus vulgaris,** acantholysis involves the layer of cells just above the basal layer giving rise to a suprabasal blister.
- In **pemphigus foliaceus,** acantholysis involves the superficial epidermis at the level of stratum granulosum.
- **Variable superficial dermal infiltration** by lymphocytes, histiocytes and eosinophils accompanies the acantholysis.

2. **Bullous pemphigoid**
   - Affects elderly people, presents with bullous lesions on normal or erythematous skin and mucosa; the bullae are tense and filled with clear fluid.
   - Usual sites are inner aspect of thighs, flexor surface of forearms, axillae, groin and lower abdomen.

   ***Pathogenesis*:**
   - It is an autoimmune disorder in which the characteristic finding is linear deposits of IgG antibodies and complement in the basement membrane zone.
   - Area affected is the basal cell-basement membrane attachment (haemidesmosomes), where the bullous pemphigoid antigen (BPAG) is located. This protein is normally involved in dermoepidermal bonding.
   - IgG autoantibodies to haemidesmosome components fixes complement with subsequent tissue injury.

   ***Morphology*:**
   - Characterized by a **subepidermal nonacantholytic blister.**
   - Lesions show perivascular inflammation (lymphocytes, eosinophils and occasional neutrophil), superficial dermal oedema and associated basal cell liquefaction, which eventually gives rise to the blister.
3. **Dermatitis herpetiformis**
   - Affects predominantly males in the 3rd and 4th decades.
   - May be associated with gluten-sensitive enteropathy (celiac disease).
   - Urticarial plaques and vesicles are seen in a bilaterally symmetrical distribution on the extensor surface of elbows, knees, upper back and buttocks.

   ***Pathogenesis*:**
   - Presence of IgA antibodies to dietary gluten.
   - Antibodies cross react with reticulin (a component of fibrils that anchor the epidermal basement membrane to the superficial dermis).
   - Resulting injury produces a subepidermal blister.

   ***Morphology*:**
   - Formation of microabscesses (fibrin and neutrophils at the tips of dermal papillae).
   - Basal cells show vacuolization and focal dermoepidermal separation, eventually leading to formation of a subepidermal bulla.
   - Direct immunofluorescence shows discontinuous, granular deposits of IgA localized in the tips of dermal papillae.

Q. Write briefly on seborrheic keratosis.

Ans. It is a common epidermal tumour that occurs most frequently in middle-aged and older individuals, usually on the trunk, extremities, head and neck.

Pathogenesis:
- Presence of activating mutations in the fibroblast growth factor (FGF) receptor 3.
- Onset of lesions may be part of a paraneoplastic syndrome **(sign of Leser–Trélat).**
- Patients may have internal malignancies, which produce growth factors that stimulate epidermal proliferation.

Morphology:
- It is a raised, pigmented lesion with a verruca-like surface, which histologically exhibits hyperkeratosis, papillomatosis, entrapment of keratin in the epidermis (horn cysts) and proliferation of basaloid (basal cell-like) cells showing increased pigmentation.

Q. Describe the clinicopathological features of squamous cell carcinoma (SCC).

Ans. SCC may present as:

1. Crusted or scaly patches on the skin with a red, inflamed base, or
2. A growing tumour, or
3. A nonhealing ulcer.

Salient features of SCC:

- SCC generally occurs in sun-exposed areas amongst people over age 50.
- May also occur on the lips, inside the mouth, on the genitalia or anywhere on the body.
- It is known to be associated with long-standing inflammation of the skin.

***Risk factors*:**

- Excessive radiological exposure (X-rays)
- Exposure to arsenic and industrial carcinogens (tar and oils)
- Exposure to ultraviolet radiation (produces DNA damage)
- Chronic immunosuppression by chemotherapy or organ transplantation (reduces host surveillance and increases susceptibility to infection by oncogenic viruses)
- Chronic nonhealing ulcers and burn scars (**Marjolin ulcer**)

***Pathogenesis*:**

- Malignant transformation of normal epidermal keratinocytes is the hallmark of SCC. The critical pathogenic event is the development of apoptotic resistance through functional loss of TP53, a tumour suppressor gene.
- UV radiation causes DNA damage through the creation of pyrimidine dimers, a process known to result in genetic mutation of TP53.
- TP53 mutations are seen in a large number of skin cancers, as well as most precursor skin lesions, suggesting that loss of TP53 is an early event in the development of SCC.
- Other genetic abnormalities believed to contribute to the pathogenesis of SCC include mutations of BCL2 and RAS, alterations in intracellular signal transduction pathways involving epidermal growth factor receptor (EGFR) and cyclooxygenase (COX) and mutations in DNA repair genes.

***Morphology*:**

- Squamous cell carcinoma in situ (CIS), sometimes referred to as **Bowen disease**, is a precursor to invasive SCC. This lesion is characterized by nuclear atypia, frequent mitoses, cellular pleomorphism and a disorganized progression of cells from the basal to apical layers of the epidermis.
- **Actinic keratosis (AK),** a similar precancerous skin lesion, is a scaly, crusty lesion in fair-skinned people, which occurs due to solar damage.
- Invasive SCC is differentiated from CIS and AK, based on invasion of the basement membrane by malignant cells seen in the former. In invasive SCC nests of malignant cells are found in the dermis, surrounded by an inflammatory infiltrate.
- Conventional SCCs can be divided into three histological grades, based on the degree of differentiation (resemblance to normal squamous epithelium), nuclear atypia and keratinization.
- A **well-differentiated SCC** (Fig. 22.1) is characterized by cells with near normal-appearing nuclei and abundant cytoplasm with **extracellular keratin pearls.**
- In contrast, a **poorly differentiated SCC** shows a high degree of nuclear atypia with frequent mitoses, a greater nuclear-to-cytoplasmic ratio and less keratinization. Poorly differentiated SCCs have an increased rate of metastasis and an overall worse prognosis.
- **Moderately differentiated SCCs** exhibit features between well-differentiated and poorly differentiated lesions.
- Histological variants include **acantholytic (adenoid) SCC**, which is characterized by a pseudoglandular appearance due to necrosis in the centre of tumour nests and **spindle cell SCC**, which has atypical spindle-shaped cells, resembling a sarcoma. Both the variants exhibit a more aggressive clinical course.

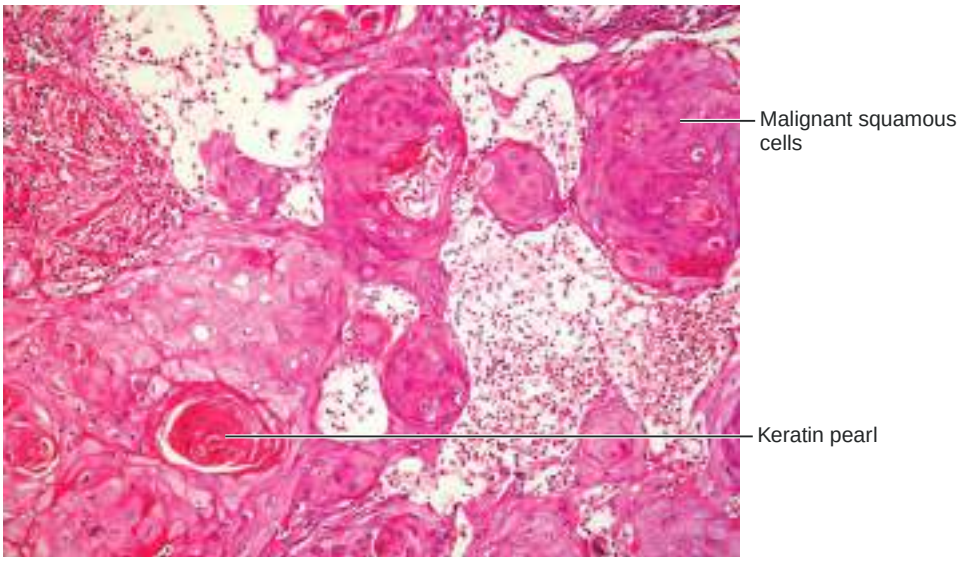


FIGURE 22.1. Section from a well-differentiated SCC showing atypical cells with abundant cytoplasm and extracellular keratin pearls (H&E; 200×).

Q. Describe the clinicopathological features of basal cell carcinoma (BCC).

Ans. BCC arises from the basal layer of the epidermis and constitutes approximately 80% of all nonmelanoma skin cancers.

- The tumour most often affects individuals aged 40–60 years; is locally aggressive and rarely metastasizes.
- Advanced lesions may ulcerate and locally invade into the underlying bone and facial sinuses like a rodent (therefore also called rodent ulcer).
- BCC is commonly located on the face, on the inner aspect of the nose, around the orbit and on the upper lip (sun-exposed parts of the body), where it appears as an insidious, painless, nonhealing ulcer or raised nodule containing a central crater.
- In patients with recurrent or deeply infiltrative tumours, involvement of the facial nerve or branches of the trigeminal nerve may be seen.

***Pathogenesis*:** Risk is related to skin type and the degree of exposure to sunlight, particularly UVB radiation. Mutations in protein patched homolog-1 (PCTH)-1 tumour suppressor gene are implicated in both sporadic and inherited forms of BCC. P53 mutations are seen in 40–60% of BCCs.

***Genetic syndromes involving BCC*:**

1. **Xeroderma pigmentosa** is a rare, autosomal recessive disorder characterized by hypersensitivity to UV radiation. It is due to defects in DNA repair mechanisms and results in predisposition to cutaneous cancers (eg, BCC, SCC and melanoma).
2. **Nevoid basal cell (Gorlin) syndrome** is an autosomal dominant disorder associated with multiple BCCs, odontogenic keratocysts, calcification of falx cerebri and rib abnormalities.
3. **Epidermodysplasia verruciformis** is an autosomal recessive disorder characterized by the development of BCC and SCC from warts.

***Types*:** Different clinicopathological types of BCC exist, each with distinct biologic behaviour:

- **Nodular or noduloulcerative BCC**
  - Constitutes more than 60% of BCCs
  - Presents as a well-circumscribed, dome-shaped, pearly nodule with or without ulceration

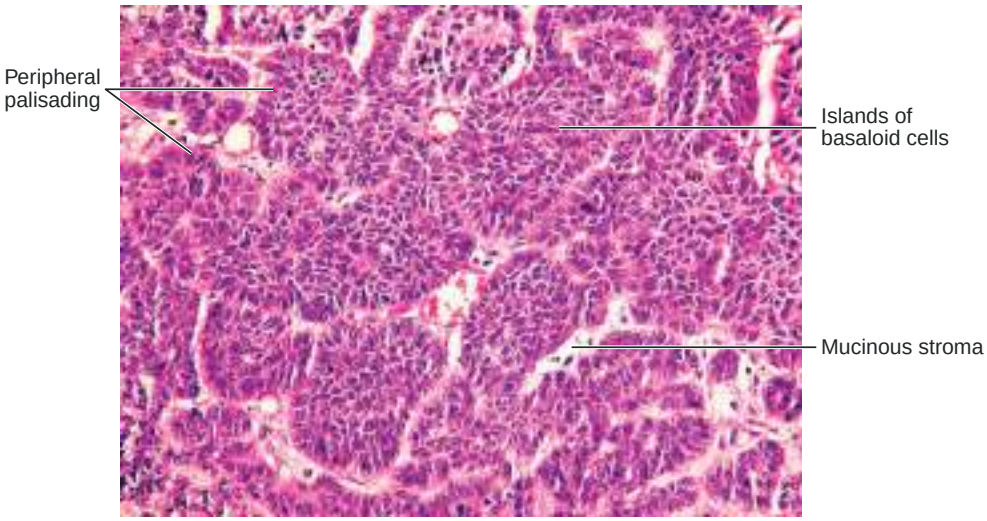


FIGURE 22.2. Section from BCC skin showing islands of basaloid tumour cells showing prominent peripheral palisading (H&E; 200×).

- **Superficial BCC**
  - This BCC subtype appears as a red scaly patch that resembles chronic dermatitis; is predominantly seen in the extremities.
  - It spreads superficially and can involve a large surface area.
- **Morphea type or sclerosing BCC**
  - Accounts for 10% cases.
  - Presents as a flat or slightly depressed, fibrotic and firm lesion.
  - It is deeply infiltrative in character and tends to extend beyond the clinically obvious margins.
- **Micronodular BCC**
  - Manifests as a plaque-like indurated lesion with poorly demarcated contours.
  - Has a high incidence of recurrence and an aggressive behaviour.
- **Other types include** keratotic BCC, infundibulocystic BCC, follicular BCC and pleomorphic BCC.

Morphology **(Fig. 22.2):**

- Tumour cells are basaloid and predominantly arranged as islands showing prominent peripheral palisading; at places forming cords and nests.
- Cells within the centre of the epithelial islands appear syncytial (having ill-defined cytoplasmic margins).
- The stroma shows varying amounts of collagen deposition with abundant mucin. A characteristic retraction artefact or clefting is exhibited by the stroma immediately adjacent to the islands/nests of tumour cells on H&E staining.

Q. Enumerate the common cystic disorders of skin.

Ans. Common cystic disorders of skin include:

1. **Epidermal inclusion cyst:** It is derived from the epidermis of a hair follicle and contains laminated keratin material.
2. **Pilar (sebaceous) cyst:** Most commonly located on the scalp, it is similar to an epidermal inclusion cyst except for the absence of a stratum granulosum layer in the cyst wall and absence of laminated keratin within the cyst.

Q. Describe the clinicopathological features of melanocytic disorders of the skin.

Ans. Melanocytic disorders of the skin include

1. **Vitiligo:** Characterized by acquired depigmentation resulting from autoimmune destruction of melanocytes.

2. **Acanthosis nigricans:** A pigmented skin lesion commonly present in the axillae that may be a phenotypic marker for an underlying adenocarcinoma of the stomach.
3. **Freckles:** Pigmented macular lesions that occur in sun-exposed areas of the skin; they are not premalignant and have a normal number of melanocytes along the basal cell layer but increased melanin within individual melanocytes.
4. **Lentigo simplex:** It is similar to a freckle, except there are increased numbers of melanocytes along the basal layer as well as increased melanin in each melanocyte.
5. **Nevus:** This denotes any congenital lesion of the skin, which has 'nevus cells'. Nevus cells are similar to melanocytes but differ from melanocytes in being arranged in clusters or nests.
6. **Melanocytic nevus:** It is a benign neoplastic proliferation of neural crest-derived melanocytes.
7. **Junctional nevi:** Contain nests of pigmented nevus cells proliferating along epidermodermal junction (appear as flat, pigmented lesions).
8. Junctional nevi usually develop into **compound nevi,** as nevus cells extend into the underlying superficial dermis, forming cords and columns of cells; so that both a junctional and an intradermal component is present (raised, pigmented and verruca-like lesions).
9. **Intradermal nevus,** which is the most common type of nevus in adults, is located in the upper dermis.
10. **Dysplastic nevi** may be a part of the **dysplastic nevus syndrome**; and they may be precursor lesions of malignant melanoma.

Malignant Melanoma

- It is a malignant tumour derived from melanocytes.
- Both sexes are affected equally; it is more common in whites than in African-Americans, and has a predilection for fair-skinned people.
- Exposure to excessive sunlight at an early age is the single most important predisposing risk factor. Other risk factors include a history of severe sunburn, dysplastic nevus syndrome, melanoma in a first- or second-degree relative and xeroderma pigmentosum.
- About 10–15% melanomas have genetic abnormalities. Most common aberrations are mutations in CDKN2A, RB and PTEN genes. Activating mutations in NRAS and BRAF are also implicated.
- Symptoms such as bleeding, itching, ulceration and pain in a pigmented lesion warrant evaluation. The following signs are indicative of development of malignancy in a preexisting lesion:
  - **Asymmetry:** One half of the lesion does not match the other half.
  - **Border irregularity:** The edges are ragged, notched or blurred.
  - **Colour variation:** Pigmentation is not uniform and may display shades of tan, brown or black; white, reddish or blue discolouration is of particular concern.
  - **Diameter:** A diameter greater than 6 mm is characteristic, although some melanomas may have smaller diameters; any growth in a nevus warrants an evaluation.
  - **Evolving:** Changes in the lesion over time are characteristic; this factor is critical for nodular or amelanotic (nonpigmented) melanoma, which may not exhibit the classic criteria above.
- Most variants have an initial **radial growth phase** in which malignant melanocytes proliferate laterally within the epidermis, along the dermoepidermal junction or within the papillary dermis; metastasis cannot occur in this phase.
- There may be a **vertical growth phase** in which malignant cells penetrate the underlying reticular dermis; metastasis can occur in this phase.
- Tumour cells are polygonal to spindled, larger than normal melanocytes, have atypical nuclei showing irregular contours and prominent eosinophilic nucleoli. Intracytoplasmic melanin is usually seen. Tumours not showing pigment are called amelanotic melanomas (Fig. 22.3). Various patterns of growth of tumour cells may be seen including solid sheets, islands, glands, etc.
- The **superficial spreading melanoma** is the most common type and primarily affects women over 50 years of age. The lower extremities and back are the most common locations. Histologically, it is characterized by pagetoid infiltration of the epidermis by atypical melanocytes.

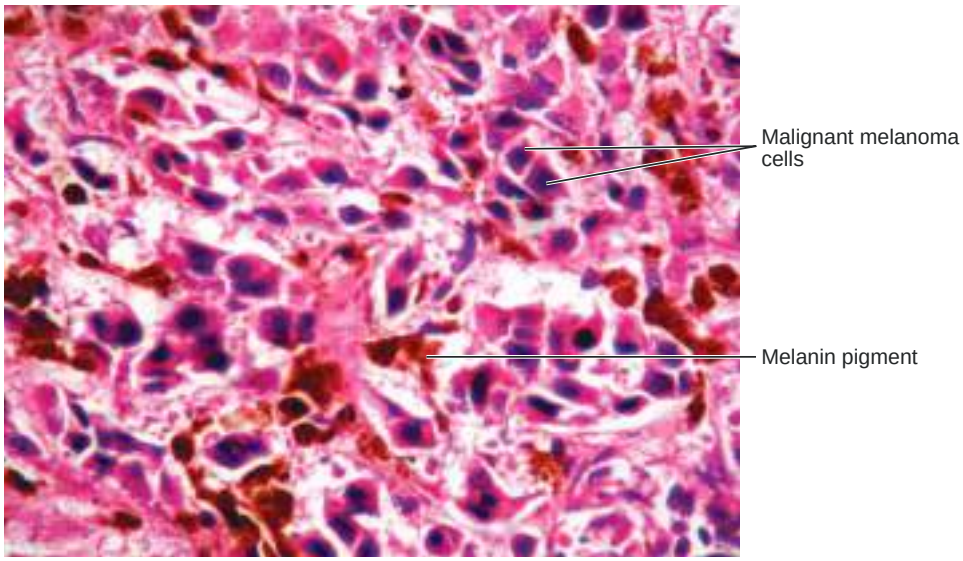


FIGURE 22.3. Photomicrograph of a malignant melanoma showing polygonal to spindled tumour cells having atypical nuclei with prominent eosinophilic nucleoli and abundant intracytoplasmic melanin (H&E; 400×).

- **Lentigo maligna melanoma** is an extension of a lentigo maligna (intraepidermal lesion) and primarily occurs on the sun-exposed face of an elderly person. Histologically, it is characterized by a predominantly junctional proliferation of melanocytes and extension along adnexal structures. Solar elastosis is typically present.
- **Nodular melanomas** lack a radial growth phase and directly invade the dermis.
- **Acral lentiginous melanomas** are located on the palms, soles or subungual regions and usually affect African-Americans.
- The **Breslow system** of staging measures the depth of invasion from the outermost granular layer to the deepest margin of the tumour; lesions with <0.76 mm of invasion usually do not metastasize; whereas, those >1.7 mm of invasion have the potential for lymph node metastasis. The **Clark system** subdivides invasion into levels I through V.

23

The Central Nervous System

NORMAL CELLS OF THE CENTRAL NERVOUS SYSTEM (CNS) AND THEIR CELLULAR MORPHOLOGY (FIG. 23.1)

Neurons

- They are organized as aggregates (nuclei and ganglia) or elongated columns/layers (eg, grey column of spinal cord or six-layered cerebral cortex).
- They have a cell body (perikaryon), a large eccentric nucleus, prominent nucleolus and abundant Nissl substance.

Glial Cells

- They form the supporting system for neurons and their dendritic and axonal processes.
- They play a role in inflammation, repair, fluid balance and energy metabolism.

Types

1. **Macroglia**
   These are derived from neuroectoderm and are of three main types:
   (a) **Astrocytes**
   - They act like fibroblasts in response to injury (undergo hyperplasia and hypertrophy, termed '**gliosis**')
   - Astrocytic processes can be demonstrated by **PTAH (phosphotungstic acid haematoxylin)** stain. Ultrastructurally, these processes are composed of abundant intermediate filaments, mostly vimentin.
   - Long-standing gliosis results in development of '**Rosenthal fibres**', which are eosinophilic elongated globular bodies present on astrocytic processes.
   - Astrocytes are star shaped glial cells with long, highly branched processes. They occupy most of the interneuronal space and mediate metabolic exchange. They have the following sub-types:
     (i) Fibrillary astrocyte: Long, thin processes; present mainly in the white matter.
     (ii) Protoplasmic astrocyte
     - Well-defined cytoplasmic margins and pyknotic nucleus
     - Few cytoplasmic processes separated by minute spaces

     (iii) Pilocytic astrocyte: Bipolar cells with long, thin, hair-like processes Glial fibrillary acidic protein (GFAP)-positive
     (iv) Gemistocytic astrocyte
     - Soft, grey and swollen cell with eccentric nucleus/prominent nucleolus
     - Abundant bright eosinophilic cytoplasm with stout cytoplasmic processes

   (b) Oligodendrocytes
   (i) Moderate size
   (ii) Small darkly staining nucleus with a clear halo around it
   (iii) Small number of short-branched processes
   (iv) Responsible for myelination of axons
   (v) Predominant glial element in white matter
   (vi) Aggregate closely around neurons in grey matter for support function (satellite cells)

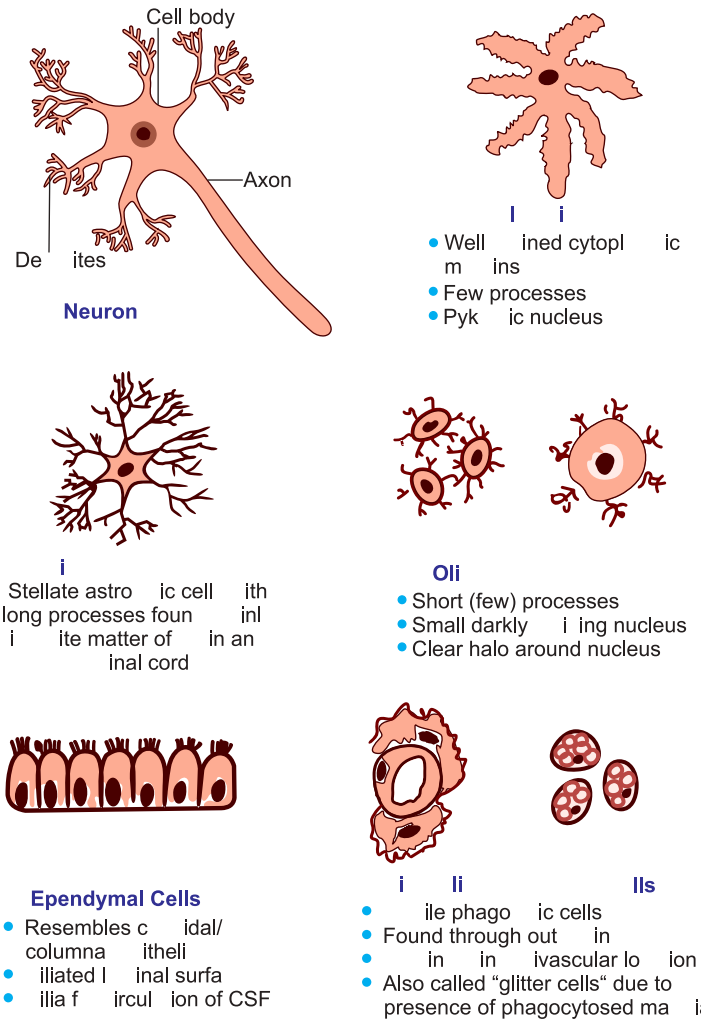


FIGURE 23.1. Normal cells constituting CNS and their cellular morphology.

(c) **Ependymal cells**
   (i) 'Epithelial-like' cuboidal to columnar
   (ii) Ciliated luminal surface (cilia for circulation of cerebrospinal fluid)
   (iii) Bases of the cells taper and breakdown into fine ramifying processes

2. **Microglia**
   (a) Small mobile cells of mesenchymal origin found throughout the brain, predominantly in perivascular location.
   (b) Irregular nuclei with little cytoplasm and tiny, highly branched processes.
   (c) In response to inflammation, they are transformed into amoeboid cells for phagocytosis.
   (d) Also called '**glitter cells**' (due to the presence of phagocytosed material).

Meninges

Leptomeninges:
The inner two meninges, arachnoid and pia mater, constitute the leptomeninges.
- ***Piamater***: Composed of fibroblasts, collagen and processes of underlying astrocytes.
- ***Arachnoid***: Lined by a layer of flattened mesothelial cells.
- ***Subarachnoid space***: Space between the two leptomeninges; contains **cerebrospinal fluid (CSF)** and major blood vessels.

Dura:
Tough fibrous covering of brain, lined on the inside by mesothelium and suspended from calvarium by denticulate ligament; encloses a space called '**epidural space**' between bone and dura.
***Subdural space*:** Enclosed between dura and arachnoid membrane; contains minute amount of fluid.

CSF

- Seventy percent of CSF is formed in the ventricular choroid plexus by a combined process of active secretion and ultrafiltration.
- Thirty percent of CSF is formed as interstitial fluid elaborated within intercellular spaces of the brain and spinal cord.
- Total volume of CSF in adults ranges between 90 and 150 mL.
- Two morphologically distinct blood-brain barriers prevent passage of plasma constituents into CSF, namely:
  1. Capillary endothelium
  2. Fenestrated choroidal capillaries enclosed by specialized ependyma found in choroid plexus epithelium
- Cell content of normal CSF is low (0–4/μL), comprising mainly lymphocytes and monocytes.
- Normal pressure of CSF is 90–180 mm of CSF (60–150 mm of water); it is measured by allowing CSF to rise in a sterile, graduated manometer tube.
- Normal CSF is crystal clear with an appearance and viscosity comparable to water.

Q. Write in detail on laboratory diagnosis of meningitis.

Ans. Inflammation of meninges is called meningitis. It is of two types:

- **Inflammation of dura (pachymeningitis):** This is usually due to extension of infection from chronic suppurative otitis media (CSOM) or fracture skull.
- **Inflammation of pia-arachnoid (leptomeningitis):** The common causes of leptomeningitis include
  1. **Infection**
     (a) **Acute pyogenic (bacterial/purulent) meningitis**
     - Infection of pia-arachnoid and of CSF enclosed in subarachnoid space
     - May extend to brain, spinal cord, optic nerves and ventricles

     ***Causative organisms*:**
     - *Escherichia coli* (infects neonates, particularly with neural tube defects)
     - *Haemophilus influenzae* (infects infants and children)
     - *Neisseria meningitides* (infects adolescents and young adults)
     - *Streptococcus pneumoniae* (infects extremes of age; common following trauma)

     ***Routes of infection*:**
     - Blood stream
     - Adjacent focus
     - Iatrogenic (during operation/lumbar puncture)

     ***Gross pathology*:** Normally clear CSF becomes turbid or frankly purulent due to pus accumulating in the subarachnoid space. Pus may interfere with normal flow of CSF leading to obstructive hydrocephalus.

     ***Microscopy*:** Numerous polymorphonuclear leukocytes, prominent around blood vessels are seen. Gram staining is done to demonstrate specific organisms.

     ***Clinical features*:**
     - Medical emergency; presents with fever, severe headache, vomiting, drowsiness, stupor, coma and convulsions.
     - Stiffness of neck on forward bending, positive **Kernig sign** (hip flexion causes pain in the knee) and positive **Brudzinski's sign** (neck flexion causes flexion of knee and hip).

***CSF findings*:**
- Cloudy/frankly turbid CSF
- Elevated CSF pressure (above 180 mm water)
- Presence of polymorphs (10–10,000/µL)
- Raised CSF protein (>50 mg/dL)
- Decreased sugar (<40 mg/dL)
- Positive bacteriologic examination (Gram stain or culture)

***Complications*:**
- Cerebral abscess formation
- Obstructive hydrocephalus
- Subdural empyema
- Cerebral infarction
- Epilepsy

(b) **Acute lymphocytic (viral, aseptic) meningitis:**
Affects children and young adults.
***Causative viruses*:** Enteroviruses, mumps, ECHO virus, coxsackie virus, EBV and HSV II
***Gross pathology*:** No distinctive change; sometimes swelling of brain
***Microscopy*:** Mild lymphocytic infiltrate in the leptomeninges
***Clinical features*:**
- Acute meningeal symptoms and fever
- Benign and self-limiting, usually ends in complete recovery
- Life-threatening complications of bacterial meningitis usually not seen

***CSF findings*:**
- Clear or slightly turbid fluid
- CSF protein normal or slightly raised
- CSF sugar normal
- CSF bacteriologically sterile

(c) **Chronic tuberculous/cryptococcal meningitis**
***Tuberculous meningitis*:**
- Affects children and adults
- Usually due to haematogenous spread (miliary tuberculosis)
- Less commonly, spreads directly from tuberculosis of the vertebral body

***Cryptococcal meningitis*:**
- Seen in debilitated or immunocompromised persons (eg, with AIDS)
- Usually due to haematogenous dissemination from a pulmonary lesion

***Gross pathology*:**
- Thick exudate in subarachnoid space, more abundant in sulci and base of brain
- Tubercles are 1–2 mm in diameter, and located adjacent to blood vessels
- Exudate in cryptococcal meningitis is scanty, translucent and gelatinous

***Microscopy*:**
- Acute and chronic inflammatory cells
- Granulomas (with or without caseation)
- AFB/capsulated cryptococci

***Clinical features*:**
- Headache, confusion, malaise and vomiting
- May have a fulminant (few weeks) or an indolent (months or years) course

***CSF findings*:**
- Mild turbidity; may form fibrin web on standing (due to increased protein including fibrinogen)
- Raised CSF pressure (>300 mm of water)
- Mononuclear leucocytosis (100–1000 cells/µL); mainly lymphocytes and macrophages
- Increased protein
- Decreased glucose
- Demonstration of AFB/*cryptococcus*

Late sequelae:
- Exudate and fibrous adhesions leading to obstructive hydrocephalus
- Tuberculous encephalitis
- Tuberculosis of the spine

The differentiating features of different types of meningitis are shown in Table 23.1.

TABLE 23.1. Differentiating features of different types of meningitis

Features	Normal	Acute pyogenic (bacterial) meningitis	Acute lymphocytic (viral) meningitis	Chronic (tuberculous) meningitis
Naked eye appearance	Clear and colourless	Cloudy or frankly purulent	Clear or slightly turbid	Clear or slightly turbid, forms fibrin coagulum on standing
CSF pressure	60–150 mm water	Elevated (>180 mm of water)	Elevated	Elevated
Cells	0–4 lymphocytes/µL	10–10,000 neutrophils/µL	10–100 lymphocytes/µL	100–1000 lymphocytes/µL
Proteins	15–45 mg/dL	Markedly raised due to • Increased permeability of blood–CSF barrier • Decreased removal of protein molecules at arachnoid level	Raised	Raised
Glucose	50–80 mg/dL	Markedly reduced due to • Impaired glucose transport • Increased glycolysis in CNS • Increased glucose utilization by WBCs and microorganisms	Normal	Reduced
Bacteriology	Sterile	Causative organism isolated	Sterile	Tubercular bacilli present

Q. Write briefly on neurosyphilis.

Ans. Neurosyphilis is syphilis affecting central nervous system (CNS). Involvement of CNS is generally seen in the tertiary stage of the disease, in approximately 10% of untreated patients.

Major Patterns of CNS Involvement in Neurosyphilis

1. **Meningeal**
   (a) Causes chronic meningitis
   (b) Histopathology shows obliterative endarteritis **(Heubner arteritis)** with perivascular inflammation rich in plasma cells
   (c) Occasionally gummas (masses rich in plasma cells) may be seen in the brain parenchyma and meninges
2. **Paretic neurosyphilis (general paresis of the insane due to invasion of brain by *T. Pallidum*)**
   (a) Insidious and progressive loss of mental and physical functions with mood alterations and dementia
   (b) Widespread individual cell death and brain atrophy
   (c) Loss of cortical neurons and glial proliferation
3. **Tabes dorsalis**
   (a) Damage of dorsal root resulting in impaired position and vibration sense, ataxia and loss of pain sensation
   (b) Loss of both axons and myelin in the dorsal roots

Q. Write briefly on viral encephalitis.

Ans. Viral encephalitis is a viral infection of the brain parenchyma, which is invariably associated with meningeal inflammation.

***Characteristic histological features*:**
- Perivascular and parenchymal mononuclear cell infiltrate
- Multiple foci of necrosis; in particular, single cell necrosis with phagocytosis of the debris **(neuronophagia)**
- Formation of glial nodules (due to glial proliferation)

***Diagnosis*:**
- Direct evidence: Presence of inclusion bodies or demonstration of organism
- Indirect evidence: Occurrence of congenital malformations (due to intrauterine viral infections) and postencephalitis Parkinsonism

***Types*:**

1. **Arthropod borne viral encephalitis:**
   ***Geographic distribution*:**
   - Eastern and Western equine, Venezuelan and St. Louis encephalitis (seen in Western hemisphere)
   - Japanese B encephalitis (seen in Far East)
   - Tick-borne encephalitis (seen in Russian and Eastern European regions)

   ***Clinical presentation*:**
   - Generalized neurological deficit
   - Seizures
   - Confusion and/or delusions
   - Stupor and coma

   ***Pathology*:**
   - Colourless CSF with increased pressure
   - Initially, neutrophilic pleocytosis followed by lymphocytosis
   - Increased CSF protein
   - Normal CSF sugar
2. **Herpes simplex virus-1-associated viral encephalitis**
   ***Salient features*:**
   - Affects children and young adults
   - Ten percent patients have history of prior herpes labialis
   - Causes encephalitis with mainly temporal lobe involvement

   ***Pathology*:**
   - Necrosis and haemorrhage
   - Perivascular inflammation
   - Cowdry Type A intranuclear viral inclusions found in both neurons and glia
3. **Herpes simplex virus-2 (HSV-2) or herpes genitalis–associated viral encephalitis**
   - Causes viral meningitis and encephalitis
   - Seen in 50% neonates born by vaginal delivery to women with active primary HSV infection
4. **Varicella zoster–associated viral encephalitis**
   Causes granulomatous arteritis and acute encephalitis
5. **Cytomegalovirus (CMV) associated viral encephalitis:** Two patient populations affected:
   - **Fetal:** Severe periventricular necrosis, brain damage, microcephaly and periventricular calcification
   - **Immunosuppressed adults:** Subacute encephalitis
6. **Rabies-associated viral encephalitis**
   ***Salient features*:**
   - Transmitted by the bite of a rabid animal usually a dog
   - Virus enters CNS from the wound site via peripheral nerves
   - Incubation period: 1–3 months
   - Nonspecific symptoms like malaise, headache and fever
   - Paraesthesias around wound, extraordinary CNS excitability, violent motor responses (convulsions), contracture of pharyngeal muscles, meningismus and flaccid paralysis

***Pathology*:**
- Intense oedema and vascular congestion
- Widespread neuronal degeneration and inflammation (most severe in the mid-brain and floor of the fourth ventricle)
- **Negri bodies** (round to oval eosinophilic intracytoplasmic inclusion bodies in pyramidal neurons of the hippocampus and Purkinje cells of the cerebellum)

7. **HIV–associated viral encephalitis**

Clinical features:
- Insidious, presents with mental slowing and mood disturbances.
- Motor abnormalities, ataxia and seizures may also be seen.

***Pathology*:**

May manifest with either of the following:
- Aseptic (lymphocytic) meningitis
- Myelin loss
- Meningoencephalitis

Q. Write briefly on Lyme disease.

Ans. It is caused by ***Borrelia burgdorferi*** (a spirochaete).

***Clinical features*:**
- Aseptic meningitis
- Facial nerve palsy
- Encephalopathy
- Polyneuropathies

***Pathology*:**

Focal proliferation of microglia with scattered organisms.

Q. Classify neoplastic lesions of CNS. Enumerate their salient clinicopathological features.

Ans. Neoplastic lesions of CNS and their Clinicopathological Features:

Incidence

- Intracranial tumours are more common than intraspinal.
- More than half are primary; rest are metastatic.
- Constitute 20% of all cancers of childhood.
- In children, majority occur in the posterior fossa, whereas in adults, the cerebral hemispheres are most commonly involved.

Unique Features of CNS Tumours:

- Benign and malignant tumours are difficult to differentiate based on morphology alone.
- Anatomic site can have lethal consequences irrespective of morphology.
- Accessibility to surgical resection is limited.
- Pattern and mode of spread are different from other malignancies (spread through CSF).

Classification

1. Primary Tumours

(a) Gliomas
  (i) Astrocytic tumours
    - Pilocytic
    - Fibrillary
    - Gemistocytic
    - Protoplasmic
    - Anaplastic
    - Glioblastoma
  (ii) Oligodendrogliomas
  (iii) Ependymomas
  (iv) Choroid plexus papillomas
  (v) Mixed gliomas

(b) Neuronal tumours
  (i) Ganglioneuromas and gangliogliomas
  (ii) Neuroblastomas
(c) Embryonal tumours
  (i) Medulloblastomas
(d) Tumours of meningeal origin
  (i) Meningiomas
  (ii) Melanomas
  (iii) Sarcomas

2. Secondary Tumours

3. Miscellaneous Tumours or Tumour-like Conditions

(a) Cysts of developmental origin
(b) Craniopharyngiomas
(c) Chordomas
(d) Dermoid cysts

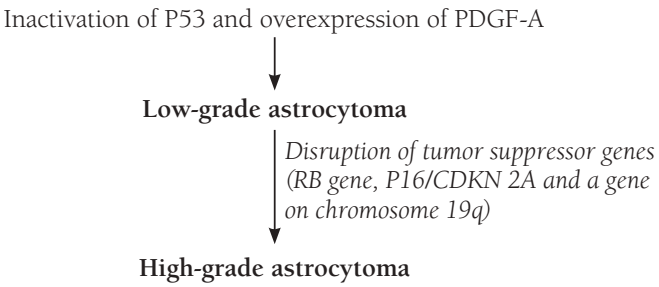
Gliomas

Grading

1. **Three-tier system**
   (a) Well-differentiated astrocytoma
   (b) Anaplastic astrocytoma
   (c) Glioblastoma
2. **Four-tier system**
   (a) Grades I–IV based on nuclear pleomorphism, mitoses, endothelial proliferation and necrosis **(WHO grading)**
   (b) Tumour grade expressed as X/IV, eg, Grade II/IV for well-differentiated diffuse fibrillary astrocytomas, Grade III/IV for anaplastic astrocytomas and Grade IV/IV for glioblastomas.

Types

1. **Astrocytomas:** Gliomas derived from astrocytes are labelled astrocytomas. They are classified into the following subtypes:
   (a) **Diffuse astrocytomas**
     (i) Constitute 80% of all primary brain tumours.
     (ii) Arise mainly from cerebral hemispheres.
     (iii) Affected age is 40–60 years.
     (iv) They show a spectrum of histological features depending on the predominant cell type (fibrillary, protoplasmic, gemistocytic, etc.)
     (v) Depending on the clinical course and outcome, they are classified into diffuse well-differentiated astrocytomas (WHO Grade II/IV), anaplastic astrocytomas (WHO Grade III/IV) and glioblastomas (WHO Grade IV/IV).
   (b) **Anaplastic astrocytomas** are tumours which show cellular pleomorphism, increased proliferation of blood vessels, necrosis and numerous mitoses.
   (c) **Glioblastoma multiforme or GM (WHO Grade IV/IV tumours)** have the following salient features:
     (i) They are the commonest gliomas in adults; usually seen in 3rd to 5th decades.
     (ii) Cerebrum is the most frequent location. Also involve septum pellucidum, basal ganglia, hypothalamus and corpus callosum **(butterfly tumours).**
     (iii) May be of two types:
       - *De novo glioblastoma*:
         - Associated with amplification of EGFR gene, MDM2 overexpression, P16 deletion and TEN mutation
         - Affects older patients
         - Has a short history (arises de novo; not from low-grade astrocytomas)
       - *Secondary glioblastoma*:
         - Molecular genetics



- Affects younger patients
- Has a long history (arises from low-grade tumours).
- Depending on the location of lesion and rate of growth; a glioblastoma may present with variably seizures, headache and focal neurological deficit.
- On gross examination the tumour appears pale yellow to salmon pink with presence of haemorrhage and necrosis. Multiple foci are seen in 7% cases (called gliomatosis cerebri). Cortex and leptomeninges may be infiltrated; the tumour may invade and spread through CSF.
- Histopathologically tumour cells show marked cellular pleomorphism. Cellular areas alternating with necrosis are seen, which may have a serpentine pattern. There is presence of primitive glial cells and multinucleate tumour giant cells. Prominent endothelial proliferation with piled up endothelial cells bulging into vascular lumina, at times, forming ball-like (glomeruloid) structures is seen. Regimentation/pseudopalisading of nuclei at the edges of necrotic foci can be demonstrated. Perivascular necrosis is common (differential diagnosis—metastatic carcinoma in which perivascular areas are spared unlike GM.
- Prognosis is bad; mean duration of survival after diagnosis is 8–10 months.

(d) **Pilocytic astrocytomas (WHO grade I/IV tumours)**
   (i) Slow growing; affect children and young adults
   (ii) Involve cerebellum, 3rd ventricle and optic nerves
   (iii) Usually cystic with a mural nodule; may be solid
   (iv) Composed of bipolar cells with long thin hair-like processes (low cellularity, low mitoses, and no infiltration of surrounding tissue)
   (v) 'Rosenthal fibres' (amorphous aggregates of GFAP) and thick-walled blood vessels can be seen

2. **Oligodendrogliomas (WHO grade II/IV tumours)**
   (a) Constitute 5–10% of all gliomas
   (b) Commonly located in cerebral white matter (frontal lobes); thalamus frequent location in children
   (c) Usually seen in adults (4th to 5th decade); less frequent in children
   (d) Slow growing; present for years, however, anaplastic oligodendrogliomas may grow into and destroy the cortex and penetrate lepto-meninges

   ***Gross pathology*:** Well-circumscribed, pink-to-red, gelatinous with foci of calcification (seen in up to 90% cases)

   ***Microscopy*:**
   - Round cells with clear cytoplasm, well-defined cytoplasmic membranes and dark nucleus (**fried-egg appearance**); grouped together in a honeycomb-like pattern
   - Anastomosing network of blood vessels
   - Endothelial proliferation unusual (unless undergoing malignant change)
   - Grade II/IV lesions. Better prognosis than astrocytomas (average survival rate is 5–10 years)

3. **Ependymomas:** Develop from lining of blood vessels and ventricles

   Site:
   (a) ***Intraneural*:** Ventricles (common location during childhood), lumbosacral spine (common location in adults) and filum terminale
   (b) ***Extraneural*:** Soft tissue of sacrococcyx

Molecular genetics: Possible association of spinal ependymomas with NF2 gene is being examined.

***Gross pathology*:**
- Well-circumscribed, typically solid papillary, friable; may show cystic areas
- Arise from the roof or floor of 4th ventricle; fill the ventricular cavity and may invade the cerebellum and medulla
- Calcification is seen in 15% of the cases.

***Microscopy*:**
- 'Epithelial-like cells' with regular round to oval nuclei and granular chromatin, dispersed in a fibrillary background (GFAP-positive)
- Gland-like/round/elongated structures called rosettes/canals
- Perivascular pseudorosettes (tumour cells arranged around vessels with thin ependymal processes directed towards the wall of the blood vessel)
- Prognosis poor despite slow growth and lack of anaplasia (attributed to frequent dissemination in CSF and poor surgical accessibility)

***Variants*:**
- Anaplastic ependymoma: Shows increased cell density, high mitotic rate, areas of necrosis, dedifferentiation (Grades III/IV histology)
- Myxopapillary ependymoma: Arises from filum terminale:
  - Composed of papillae with a core of dilated blood vessels covered by 1–2 layers of cuboidal cells. The fibrovascular stroma may show mucoid degeneration.
  - Prognosis depends on completeness of surgical resection.
- Subependymoma:
  - Diffuse proliferation of subependymal fibrillary astrocytes and ependymal cells
  - Small, multiple and symptomless
  - Usually arise in the 4th ventricle
  - Solid, sometimes calcified; if large, may lead to hydrocephalus

4. **Choroid plexus papilloma**
   (a) In adults, usually arise from the 4th ventricle and cerebellopontine angle. In children, lateral ventricles are a common location.
   (b) May cause generalized enlargement of ventricular system and subarachnoid space. Ventricular obstruction may lead to hydrocephalus.
   ***Gross*:** Cauliflower-like, soft, friable and crumbling pink mass
   ***Microscopy*:** Specialized ependymal cells recapitulate structure of normal choroid plexus.
   ***Prognosis*:** Difficult to remove and commonly recurrent

Neuronal Tumours

These are tumours containing mature-appearing neurons (ganglion cells). **Gangliocytomas** contain only neurons and **gangliogliomas** contain neurons admixed with glial cells.

Poorly Differentiated or Embryonal Neoplasms

Thought to be neuroectodermal in origin; however, rarely express, if any, phenotypic markers of mature cells of nervous system, eg, medulloblastoma and atypical teratoid/rhabdoid neoplasm.

Medulloblastoma

- Constitutes 20–25% of all intracranial tumours in children.
- Exclusively located in cerebellum (three-fourth involve midline or vermis; rest in cerebellar hemispheres). Also occurs along the cerebellopontine angle.
- Molecular genetics: Loss of material from short arm of chromosome 17 usually in the setting of an abnormal chromosome derived from duplication of the long arm of chromosome 17. The identity of the tumour suppressor gene lost not clear (Not P53).

***Gross pathology*:** Fourth ventricle compressed or invaded by an unencapsulated, grey white, soft, haemorrhagic and friable mass without cystic change, leading to hydrocephalus.

***Microscopy*:**
- Cellular tumour composed of round to oval carrot-shaped nuclei with ill-defined scanty cytoplasm.
- Tumour cells have a tendency to form linear chains, which infiltrate through cerebellar cortex and aggregate beneath pia-subarachnoid space and eventually disseminate through CSF.
- Rosettes may be centred by delicate argyrophillic fibrils of tumour cells.
- Tumour shows a prominent desmoplastic response.

***Prognosis*:**
- Highly malignant but exquisitely sensitive to radiotherapy
- Extraneural metastasis to bone and lymph nodes common

Atypical Teratoid/Rhabdoid Neoplasm

- Malignant tumour of childhood (WHO grade IV/IV)
- Common locations include posterior fossa and supratentorial compartment
- Shows divergent differentiation into epithelial, mesenchymal, neuronal and glial components
- Often shows rhabdoid cells as seen in rhabdomyosarcoma

Tumours of Meningeal Origin

Most common tumour of meningeal origin is a meningioma

Meningioma

- Constitutes 15% of all CNS tumours in adults and 3% of childhood tumours.
- Peak incidence in 5th to 6th decade; females are more commonly affected than males.
- Slow growing; develops from specialized arachnoid cells of villi that project into the lumen of dural venous sinuses.
- Common locations: Superior sagittal sinus, sphenoid ridge, tuberculum sellae, olfactory grooves, posterior cranial fossa and ventricles.
- Molecular genetics: Most common abnormality is loss of chromosome 22. Also seen are deletions in the region close to but different from 22q12 that harbours NF2 gene.

***Gross pathology*:**
- Well demarcated/unencapsulated
- Fixes to dura and buries itself in a cup-like bed; cerebrum practically never invaded
- Cut surface is whorled; haemorrhage and necrosis may be seen
- Rarely, calcification, ossification and cyst formation are observed
- Extends into muscles, air sinuses and orbit; may be associated with reactive hyperostosis of bone

***Histological types*:**
- **Syncytial:** Poorly defined polygonal cells arranged in sheets and tight groups; whorling present (Fig. 23.2).
- **Fibroblastic:** Elongated cells with abundant collagen
- **Transitional:** Overlapping features of syncytial and fibroblastic type
- **Psammomatous:** Numerous psammoma bodies due to calcification of syncytial nests of meningothelial cells
- **Secretory:** Characteristic PAS-positive intracytoplasmic droplets present
- **Microcystic:** Loose spongy appearance with microcyst formation
- **Papillary:** Papillary appearance (fibrovascular cores with pleomorphic cells around them); high rate of recurrence
- **Angioblastic:** Vascular variant of meningioma

Note: Xanthomatous degeneration, osseous metaplasia and moderate nuclear pleomorphism are common and usually of no prognostic significance in meningiomas.

Atypical meningiomas (WHO grade II/IV) are locally aggressive and have a higher rate of recurrence. Histologically they show >4 mitoses/10HPF or at least three of the following features:
1. Increased cellularity
2. High N/C ratio

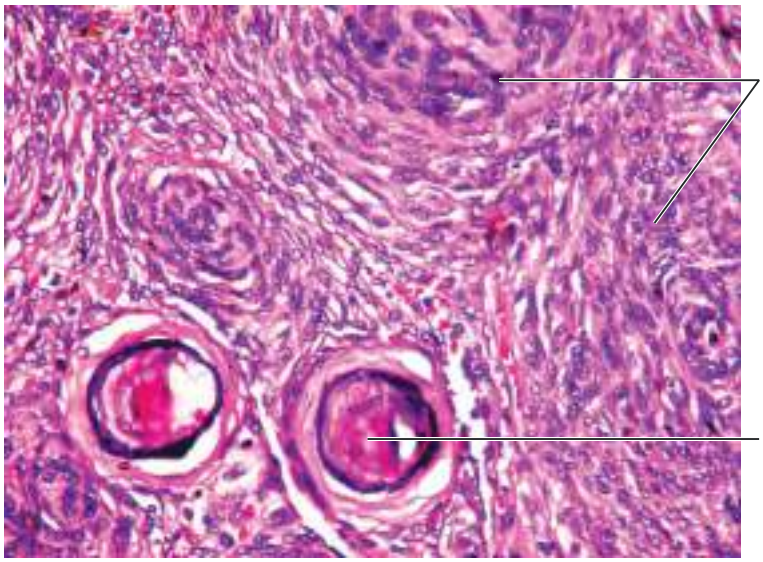


FIGURE 23.2. Section from a meningioma shows polygonal cells with ill-defined cytoplasmic margins arranged in sheets and tight groups with presence of whorling and psammoma bodies (H&E; 200×).

3. Presence of small cells
4. Prominent nucleoli
5. Necrosis

Features indicating/suggesting malignant change (Anaplastic or grade III/IV meningioma):

- Infiltration of underlying brain by a tumour with the appearance of a high-grade sarcoma with some diagnostic features of a meningioma.
- Abundant mitoses (>20 mitoses/10 HPF)
- Multifocal microscopic foci of necrosis

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